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Section B.—Biological Sciences.

On the Function of Chlorophyll.

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It has been generally assumed that the function of chlorophyll is to absorb radiant energy from light and to render this available for the production of formaldehyde from carbon dioxide and water, the formaldehyde being subsequently polymerised to carbohydrates, and oxygen being set free. This is supported by the fact that chlorophyll contains magnesium* and that in a cold watery solution of carbon dioxide this metal produces formaldehyde,† which readily polymerises spontaneously to paraformaldehyde, $C_2H_4O_2$, or metaformaldehyde, $C_3H_6O_3$, and into hexose sugars in the presence of lime-water or other weak alkali.

Usher and Priestly‡ stated that chlorophyll films when exposed to sunlight decomposed carbon dioxide, producing formaldehyde and hydrogen peroxide. The latter was responsible for the bleaching of chlorophyll in sunlight, and if a "katalase" enzyme was present the oxygen was set free and the chlorophyll remained green. "The bleaching of the chlorophyll in sunlight whether carbon dioxide is present or not is due to the formation of hydrogen peroxide." As a matter of fact chlorophyll films immersed in a solution of hydrogen peroxide remain green for a long time in darkness, and when

* A full account of the work on the chemistry of chlorophyll, by Willstätter and others, is given in the 'Chemistry of Plant Products,' by Haas and Hill, pp. 222–241 (1913).

† Fenton, 'Journ. Chem. Soc.,' vol. 91, p. 687 (1907).

‡ 'Roy. Soc. Proc.,' B, vol. 77, p. 369 (1906); vol. 78, p. 318 (1906); vol. 84, p. 101 (1911).

exposed to light do not bleach much more rapidly than similar films exposed to ordinary air.

In addition I was able to show* that formaldehyde is a direct product of the oxidation of chlorophyll when exposed to light, that none is produced in an atmosphere of pure carbon dioxide, and that when chlorophyll is exposed to light in the presence of oxygen and of carbon dioxide, oxygen is absorbed instead of being liberated. I pointed out that these observations supported the old view that chlorophyll itself is a stage in photosynthesis. Schryver† found that more formaldehyde was formed in the presence of carbon dioxide than in its absence, and suggested that any excess of formaldehyde might combine with the chlorophyll and be set free again on exposure to light. Direct tests by myself, using chlorophyll films and formaldehyde solutions kept in pure nitrogen in darkness, failed to give any support to this view. With various interruptions work in this direction has been in progress during the last five years. The appearance of an interesting paper by H. Wager, on the action of light on chlorophyll,‡ leads me to give an account of my own work up to the present time, leaving various points of detail for further papers.

Wager's conclusions are, in brief, that in sunlight, chlorophyll absorbs oxygen and decomposes into aldehydes, of which a small portion is formaldehyde, and into an oxidising agent capable of liberating iodine from potassium iodide, which is not hydrogen peroxide but may be an organic peroxide. This action takes place in the absence of carbon dioxide, and Wager inclines to the view that the production of sugar in the green leaf may be initiated by the photo-oxidation of the chlorophyll and the subsequent polymerisation of the aldehyde thus formed, rather than by the direct photosynthesis of carbon dioxide and water.

A possible objection that might be raised to these observations is that the methods of extraction given would not yield pure chlorophyll but a mixture of it with xanthophyll, carotin, lecithin, phytosterin and waxy substances. It is obviously of great importance to distinguish between the action of light upon these different substances, which can only be done by using them in pure form.

Separation and Purification of Chlorophyll, Carotin, and Xanthophyll.

The methods described by Willstätter for extracting and purifying chlorophyll were used with slight modifications. Grass leaves were immersed for

* 'Roy. Soc. Proc.,' B, vol. 80, p. 30 (1908).

† 'Roy. Soc. Proc.,' B, vol. 82, p. 226 (1910).

‡ 'Roy. Soc. Proc.,' B, vol. 87, p. 386 (1914).

1—3 minutes in boiling water. As much water as possible was then squeezed from them with a strong screw press. Sufficient absolute alcohol was added to moisten them, and within a few minutes all liquid was squeezed out in the press. The pale green liquid was thrown away. The residue was just covered with cold alcohol and digested in a closed glass cylinder in darkness in a cool chamber for at least one day. All the liquid was then poured off and squeezed out. One half of the volume of petrol ether was added, and then sufficient water to bring about separation. After standing in a large separating funnel the lower liquid was run off, then the scum between, and then the petrol ether. This was evaporated to dryness by a partial vacuum and gentle warming, redissolved in a minimum quantity of cold absolute alcohol, and again separated as before. The petrol-ether extract was allowed to stand in contact with water for some days. If any red carotin separated out it was again purified.*

This method is more satisfactory than when dried leaf meal is used, since the preliminary treatment removes a large amount of the waxy and other extractives that would otherwise be dissolved along with the chlorophyll. The separation is based upon the fact that carotin is only sparingly soluble in cold alcohol, that xanthophyll is soluble in water and in any mixture of alcohol and water, whereas carotin and chlorophyll are thrown out of an alcoholic solution by the addition of water and are readily soluble in petrol ether. Although xanthophyll is soluble in water, contact with the latter will not remove it from a petrol-ether solution of a waxy solid like chlorophyll unless sufficient alcohol is present, but pure solid xanthophyll dissolves slowly in water forming a solution which filters as a clear yellow liquid. If any carotin, wax, or chlorophyll is present, the xanthophyll dissolves in water with difficulty or not at all, owing to the lack of proper contact.

To obtain pure xanthophyll, the brownish-yellow alcoholic liquid was enriched with alcohol, shaken up again with petrol ether, and separation produced by the addition of water. After long standing in darkness the liquid beneath becomes perfectly clear and, after filtering, it may be evaporated to dryness by gentle warming and a partial vacuum in darkness. The residue was extracted with cold absolute alcohol, filtered again, evaporated to dryness, dissolved in petrol ether, and filtered. This gave a dark liquid, brownish yellow when shaken up, yellow when dilute, reddish brown by transmitted light when concentrated, but free from chlorophyll and with no red fluorescence.

Pure carotin can be obtained by treating the prepared grass material with

* A very strong solution of chlorophyll in petrol ether will always throw out some of its carotin in contact with water if any is present.

hot alcohol, separating with petrol ether, and allowing the strong chlo phyll solution to stand in contact with a little water for some days the absence of light and oxygen. Small red particles of carotin separ out, which can be collected and washed with cold absolute alcohol.

To obtain larger quantities, the cortex of carrots can be grated, rubbed with water, and then all the juice pressed out. On boiling the red liq a scum of red carotin separates. This, after washing, can be dissolved i minimum quantity of hot absolute alcohol, filtered hot, and allowed stand. Most of the carotin separates out, and all of it if a little wat is added. The residue is washed with water, then with a little d absolute alcohol, dried, dissolved in petrol ether, and filtered.

All the processes must be carried out as far as possible out of contact with the air, and in darkness or very feeble light. When prepared, the solutions of all three pigments must be kept in small tightly stoppered and completely filled bottles in darkness until they are needed. Under these circumstances all three pigments keep for a long time without appreciable change.

The "Iodoxidase" Action of Chlorophyll in Light.

Wager concluded that one of the products of the decomposition of chlorophyll in light was "an active chemical agent capable of bringing about the liberation of iodine from potassium iodide," and that it was possibly an organic peroxide and was a gas (p. 394). The action is easily obtained by tinging potassium iodide and starch paper with a little chlorophyll, moistening and exposing to light in moist air. I am not, however, able to confirm Wager's statement that blue light will liberate iodine from potassium iodide. It must be remembered that the reaction is a very delicate one, which may be produced by a variety of causes. Touching the paper with the fingers or contact with the cork of a flask may cause a blue colour to be given on exposure to light, the presence of traces of acid, of certain organic or metallic oxidases will have the same effect, as well as traces of various gases often present in the air. Certain kinds of paper on moistening with potassium iodide and starch readily turn blue in sunlight, and may even develop a weak peroxidase reaction with guaiacum. Pure, well washed Swedish filter paper is free from this defect and may be used with safety. An important point is, also, that the solutions used must be perfectly neutral. Freshly prepared alcoholic chlorophyll is faintly acid, but the purified petrol-ether solution is neutral. Films of the former liberated iodine very readily in sunlight.

Wager found that paper tinged with eosin or methyl green would also give an "iodoxidase" reaction on exposure to light. Not only is this the case,

but carotin and xanthophyll show the same reaction when exposed to light. A long series of tests showed, however—(1) that the reaction was given only when actual contact was assured with the substance undergoing oxidation; (2) that it was not due to any volatile product of decomposition; (3) that none of the products of decomposition had any power of liberating iodine from potassium iodide; (4) that partially bleached paper treated with starch and potassium iodide and then kept in darkness gave no blue at whatever stage the bleaching was stopped; and (5) that no alkalinity was produced (red litmus paper + chlorophyll + potassium iodide). This last test shows that no hydrogen peroxide is formed.

A curious point worth noting is that moist blue litmus paper bleaches much more rapidly in sunlight than red litmus paper, and that the presence of a little chlorophyll, carotin, or xanthophyll on the paper hastens the bleaching, especially with the blue litmus.

The conclusion seems justified that chlorophyll, xanthophyll, carotin, etc., when exposed to sunlight, act not merely as oxidases to themselves but may also accelerate the oxidation of other substances, if in contact with them and if the supply of oxygen is abundant. This is borne out by the fact that if a watery solution of xanthophyll with a supernatant layer of guaiacum is exposed to sunlight, a green or blue layer forms between the two liquids which soon disappears again. All these actions are only shown when an abundance of oxygen is present, as when films are exposed to air. If the potassium iodide and starch are mixed with a watery solution of xanthophyll, or with a watery emulsion of carotin or chlorophyll, no perceptible liberation of iodine takes place on exposure to light. In the case of chlorophyll films or of carotin and xanthophyll films on filter paper, the possibility of traces of liberated potash combining with the chlorophyll or filter paper would naturally favour the liberation of appreciable amounts of iodine.

There is, therefore, no evidence that peroxidases, either organic or inorganic, are among the products of the decomposition of chlorophyll by oxygen and sunlight. The "iodoxidase" reaction in this case merely indicates that oxidation has been taking place. A slight liberation of iodine may even take place on a dry filter paper impregnated with chlorophyll and potassium iodide and then bleached, a blue colour showing on moistening.

The Oxidation of Chlorophyll.

Dry chlorophyll films will bleach fully when exposed to sunlight in dry air, but usually take five to ten times as long as similar films in moist air. If the chlorophyll film is at all thick, the bleaching is greatly retarded by the fact that the colourless products of oxidation form a waxy film on the

surface, which is almost impermeable to oxygen. Thus, in one case, 0·73 grm. of dry chlorophyll (petrol-ether extract), after one week's exposure in dry CO_2-free air, was partly bleached and weighed 0·4 grm. For full bleaching four months' exposure was necessary, the weight then being 0·2 grm., and after extraction with water and drying being 0·13 grm. of white insoluble waxy solid.

Filter paper saturated with chlorophyll is unsuitable for exact experiments, and in addition moist filter paper after long exposure to sunlight may develop appreciable traces of reducing sugar, which can be extracted by water. Hence thin sheets of glass and mica were used on which solutions of chlorophyll in petrol ether were allowed to evaporate and form even films. For certain experiments thin-walled glass tubes 6 feet in length were employed.

To determine the influence of carbon dioxide on oxidation, dry films were exposed to sunlight in nearly dry air with and without carbon dioxide. In the presence of a little carbon dioxide the bleaching appeared to be accelerated and 0·44 and 0·453 grm. of chlorophyll left 0·198 and 0·22 grm. of white residue, whereas in the absence of carbon dioxide 0·462 and 0·43 grm. of chlorophyll left 0·145 and 0·123 grm. of dry white residue. This residue contains a little matter soluble in water and this is slightly more abundant in the films oxidised in the presence of carbon dioxide and the weight of the residue is greater. Dry films oxidised in dry air contain relatively little matter soluble in water.

Tubes were then lined with dry chlorophyll, filled with pure dry nitrogen and pure dry carbon dioxide and exposed to light for periods of 1 to 8 weeks. All remained green, but the films in CO_2 become a little more yellowish, and a little of the CO_2 disappeared. No oxygen or formaldehyde was formed. Similar tubes filled with dry air were exposed until bleaching ceased. The residual gas contained no oxygen but 2·2 per cent. of gas removable by potash. Further tests showed that this gas consisted wholly of formaldehyde gas.

Additional evidence that chlorophyll is able slowly to combine with CO_2 during its oxidation was obtained by exposing thick films to sunlight in air containing a little moisture and carbon dioxide until fully bleached, which took just over a month. In one case 0·8 grm. left a dry bleached residue of 0·785 grm. dry weight, whereas in pure air the bleaching was much slower and a similar quantity lost 68 per cent. by weight. A portion of this may be due to the fact that in pure dry air a slow loss of weight by photo-oxidation continues for some time in the bleached residues, whereas if the film is thin and the oxidation rapid in moist air, there may be little or no loss of weight by the time the film is bleached, the oxygen absorbed weighing nearly as much as the gas lost. Thus 0·49 grm. of chlorophyll spread out in a thin film

on mica sheets and oxidised rapidly in moist air and sunlight weighed when dried 0·478 grm. Possibly some water may also enter into combination.

The Gaseous Products of the Photo-oxidation of Chlorophyll, Carotin and Xanthophyll.

Thin-walled glass tubes 6 feet in length were warmed, and a strong solution of chlorophyll in petrol ether poured in. The heavy vapour falls out of the tube, and with a little practice a film of chlorophyll of even thickness can be left lining the tube. These were exposed to sunlight until bleached (usually 3 to 5 days) in a stream of moist CO_2-free air, which was led into water through a fine capillary exit tube. In one case where four lengths of six foot tubes were used 1·14 grm. of chlorophyll left 1·19 grm. of residue, while the water contained no CO_2, but smelt strongly of formaldehyde and, by Schiff's test, comparing with standard strengths, contained 0·32 grm. of formaldehyde. On evaporating to dryness, it left a white residue of paraformaldehyde equivalent to 0·14 grm. from the total solution. No other aldehyde was present in the solution or in the residue in the tube.

Similar experiments carried out with tubes lined with pure carotin and xanthophyll films showed that during the photo-oxidation of these substances, no carbon dioxide, but formaldehyde gas is produced, and in the case of xanthophyll possibly a little water vapour is also formed.

The question at once arises as to whether chlorophyll, like lime-water and feeble alkalies, has the power of polymerising formaldehyde to reducing sugars. If equal quantities of (*a*) water and (*b*) a watery solution of formaldehyde were added to an equal volume of an alcoholic solution of chlorophyll and the mixture exposed to sunlight in the presence of oxygen, (*b*) turned brown while (*a*) was still green, but on long exposure (*a*) became quite pale while (*b*) was still a darker brown. The liquids were then filtered and evaporated to dryness and gently heated, until in the case of (*b*) no smell of formaldehyde was perceptible and the loss of weight ceased. The residues and the dry residues from filtering were weighed.

	Insoluble residue.	Soluble solids.
A	0·31	0·81
B	0·425	0·925

In both cases the soluble solids gave a strong reduction with Fehling's solution. Alcoholic chlorophyll, however, sometimes contains traces of reducing sugar, and in this experiment carbon dioxide was not excluded.

Hence, in a second experiment, carbon dioxide was excluded, and of two

flasks (A and B) containing 40 c.c. of dilute formaldehyde, one also contained a film of 0·1 grm. of chlorophyll (A). When this was bleached the filtrates were evaporated to dryness on a water-bath, weighed, and then further heated to drive off the paraformaldehyde.

	First dry residue.	Final dry residue.
A	1·24	0·15
B	1·25	0·052

The final dry residue from A gave a strong reduction with Fehling's test. Since the bleached residue in A still weighed 0·085 grm., there is apparently evidence here of a feeble polymerisation of formaldehyde by chlorophyll.

The Solid Products of the Photo-oxidation of Chlorophyll, Carotin and Xanthophyll.

In all three cases the solid residues consist of a portion soluble in water, and of a white waxy solid insoluble in water but melting when heated. The latter appears to consist, in the case of carotin, partly at least of phytosterin.*

The white insoluble residue from the bleaching of chlorophyll is a waxy solid with a smell of beeswax. In one experiment 0·4 grm. yielded 1·99 per cent. of magnesium oxide (pure chlorophyll should yield 4·5 per cent.), so that apparently after decomposition some of the magnesium can be removed by washing with water. The white "chlorophyll wax" melts gradually on warming, like sealing wax, beginning about 80° C., and is rather brittle in mass when cold. Possibly it consists of a mixture of substances. It dissolves readily in hot alcohol, very sparingly in cold alcohol, and is moderately soluble in petrol ether.

The watery extract from fully bleached chlorophyll contains no aldehydes. It has a bitter taste, turns turbid on boiling or on adding copper sulphate, but becomes clear on adding sodium hydrate, and gives a strong reduction with Fehling's test. On warming with a drop of sulphuric acid, the white precipitate thrown down forms an oily solid clinging to the tube, and on filtering the clear solution gives a strong reduction with Fehling's test. In one experiment 0·61 grm. of chlorophyll yielded 0·64 grm. of dry bleached residue after full oxidation in moist air deprived of carbon dioxide, of which 0·23 grm. was removed by cold water, and 0·41 grm. remained as an insoluble film. If the film is properly prepared and the contact with water made gradually, none of the film breaks away from the glass or mica.

The tendency to turbidity on the part of the watery extract prevents

* See Czapek, 'Biochemie der Pflanzen,' p. 172.

examination with the polariscope. Accordingly large films on mica and glass sheets were placed vertically in gas cylinders filled with air and kept saturated with water vapour. The lower pointed end of the sheet rested in a small beaker. After some days' exposure to sunlight a few drops of a gummy liquid collected in the beaker. This has a sweetish taste, a faint brown colour, forms a clear solution with water, and gives a strong reduction from Fehling's. The liquid gave the glucosazone test, and with phenylhydrazin, sodium acetate, and alkali, a red colour with a faint violet tinge on warming. The last test must always be done with a blank control, since on long standing a slight red colour may be given in the absence of sugar. Distinct traces of reducing sugar were also given by the watery extracts from the white residues from xanthophyll, but no reduction or a doubtful trace with the watery extract from bleached carotin, bleached in moist CO_2-free air. No sugar at all could be obtained from similar films kept in darkness.

Apparently both dextro- and lævo-rotatory sugars are formed. Thus after bleaching 0·72 grm. of chlorophyll in moist CO_2-free air, the watery extract gave a doubtful dextro-rotation indicating not more than 0·003 grm. of sugar, whereas a Fehling's estimation on Soxhlet's method indicated 0·18 grm. of reducing sugar. During the oxidation of xanthophyll films, as much or even more sugar appears to be formed as during the oxidation of chlorophyll. If to an alcoholic solution of xanthophyll half its volume of water is added, and the solution evaporated to dryness after bleaching in the presence or absence of carbon dioxide, the filtered watery extract gives a strong reduction with Fehling's. The bleaching may be hastened by the addition of hydrogen peroxide, but not if a subsequent test is to be made for sugar.

The Photo-oxidation of Xanthophyll, $C_{40}H_{56}O_2$.

In a preliminary experiment the alcoholic extract from the dried separation residues was used and lined 18 feet of glass tubing. Full bleaching took two days; moist air free from CO_2 was used. The water in which the gas was collected smelt strongly of formaldehyde and gave a strong pink with decolorised magenta. It contained no carbon dioxide. The dry residues in the tubes weighed 0·427 grm. (from 0·46 grm. of dry xanthophyll). The watery extract from the residue gave a strong reduction with Fehling's test but contained no formaldehyde or other aldehydes. The white waxy residue insoluble in water appeared to resemble the residue from the oxidation of carotin.

Subsequent tests showed, however, that xanthophyll prepared in this way may contain a trace of sugar and apparently some phytosterin. Accordingly the alcoholic extract was evaporated to dryness, dissolved in a minimum of

cold absolute alcohol, filtered, again evaporated to dryness, and dissolved in petrol ether, in which the xanthophyll is more soluble and keeps better than in water or even in alcohol.

Using 18 feet of tubing lined with 0·38 grm. of xanthophyll, the bleaching was completed after 12 hours' exposure to sunlight. The water in the collecting tube contained 0·155 grm. of formaldehyde. Some of the formaldehyde may escape solution, however, since the collecting tube always smells stronger of formaldehyde than does a solution of equal strength as indicated by colour tests. The colourless residue in the tubes weighed 0·37 grm., and the whole of it came away in water, forming a slightly turbid liquid, which gave a dense white precipitate with a drop of sulphuric acid. On boiling a pale yellow oily solid separated out on the sides of the tube, the liquid filtering clear. It showed a doubtful feeble dextro-rotation equivalent to 0·001 grm. of glucose (0·01 per cent. in 10 c.c.), and gave a strong reduction with Fehling's equivalent to 0·24 grm. of glucose.

If these numbers are correct they would indicate an absorption of 38 per cent. by weight of oxygen, or of oxygen and water vapour. Haas and Hill quote (p. 238) an absorption value of oxygen of 36·55 per cent. for xanthophyll. In my own experiments the weight and surface of the glass was necessarily relatively so great as to preclude very exact weighing.

The Photo-oxidation of Carotin.

This, according to Arnaud, has the formula $C_{26}H_{38}$, and absorbs up to 21 per cent. by weight of oxygen, whereas Willstätter gives it the formula $C_{40}H_{56}$ and an absorption value for oxygen of 34·3 per cent.* Carotin oxidises much more rapidly than either xanthophyll or chlorophyll. Thus, using thin films of equal weight spread over the same area and exposed to full sunlight, carotin took one hour, xanthophyll 12 hours, and chlorophyll 20 hours to bleach completely. With very thin films, however, there is less relative difference in the rate of bleaching, while with thick films the difference in the relative rates increases. A thick film of chlorophyll may take months to bleach fully. This is largely a matter of the permeability of the oxidised surface layers to oxygen. During the oxidation of chlorophyll the blue-green colour passes to green, then yellow (or brown if a thick layer), then colourless. During the yellow stage xanthophyll appears to be set free, and subsequently to be oxidised, but no carotin.

In one experiment with carotin obtained during the purification of chlorophyll, 0·3 grm. films of carotin were exposed in tubes to light in a current of moist CO_2-free air. The dry colourless transparent residue

* For literature, see Haas and Hill, as well as Czapek's 'Biochemie.'

weighed 0·26 grm. It turned white in water, but was wholly insoluble in water and contained no sugar. The gas from the tubes was passed into water, which gave a distinct pink with Schiff's test. Apparently oxidised carotin produces formaldehyde but no sugar or carbon dioxide. The amount appears to be less than with chlorophyll or xanthophyll, but further quantitative investigations are necessary on this point. The supply of carotin obtained from the chlorophyll extraction was soon exhausted as the yield is very small, but carotin extracted from carrots may be used in its place. It also appeared to give off appreciable quantities of formaldehyde gas during bleaching, while the bleached residue was practically insoluble in water, and the watery extract either gave no reduction at all, or a doubtful trace with Fehling's test.

The Reduction of Xanthophyll to Carotin.

Although the former differs from the latter simply in containing two atoms of oxygen, it is less readily oxidised, and no oxidising agency tried was found to convert carotin into xanthophyll. According to Palladin, however,* carotin is converted by an oxidase into xanthophyll, while a reductase enzyme carries out the reverse change. Using a variety of plant oxidases, including those of the carrot, apple, potato and parsnip, I have not been able to produce any conversion of carotin into xanthophyll using finely divided carotin and watery or glycerine oxidase extracts. Under water in fact carotin only oxidises slowly and with difficulty even when exposed to sunlight.

The conversion of xanthophyll into carotin is, however, readily produced by adding magnesium dust or zinc dust to a watery solution of xanthophyll in darkness. In the first case nascent hydrogen acts as the reducing agent and the action is rapid, being completed in one to a few hours. The zinc acts slowly (2—3 days). On filtering, a clear liquid comes through, and after washing the residue with absolute alcohol, petrol-ether dissolves out the carotin, which when evaporated is insoluble in water, and has the usual properties.

The Combination of Carbon Dioxide with Chlorophyll.

Tubes were lined with dry chlorophyll, sufficient water added to cover the film, and a current of carbon dioxide passed through until the water was saturated. The tubes were then drawn out and sealed while the gas was passing through, leaving half the tube filled with gas. On exposing to sunlight the rise of temperature causes bubbles of gas to separate on the film, distorting it and making it form an irregular network adhering to the glass. The bright green colour is lost. After a week the gas above had decreased

* 'Ber. d. D. Bot. Ges.,' vols. 26A and 27 (1908 and 1909).

38 per cent. in volume to 80 c.c. It contained 79·7 c.c. of carbon dioxide and 0·3 c.c. of nitrogen.* On adding cold alcohol to the film it formed a pure yellow solution of xanthophyll, leaving a slight white insoluble portion on the glass. The water from the tube contained no perceptible amount of formaldehyde or of reducing sugar.

A similar experiment was repeated with a large tube with exactly similar results, the residual gas being less in amount and consisting of 99·8 CO_2 and 0·2 per cent. nitrogen. The yellow film when dried weighed 0·44 grm., whereas the original chlorophyll weighed 0·34 grm.

Evidently, therefore, chlorophyll, when exposed to sunlight in the presence of carbon dioxide and water, increases in weight and forms xanthophyll and a colourless waxy solid, but liberates no free oxygen.

The same change takes place in darkness, but more slowly, and in the same way the oxidation of chlorophyll takes place slowly in darkness, although it is very greatly accelerated by exposure to light.

In a second experiment a film of dry chlorophyll weighing 0·174 grm. was exposed to air in darkness for 10 days. It lost 0·006 grm. of weight, and, during the next seven days, 0·004 grm. The next week it was kept in a mixture of dry air and carbon dioxide. It lost a further 0·003 grm. in weight but was still quite green. During the next week it was kept still in darkness, but in water saturated with CO_2. The film became dull, contained mainly xanthophyll with a trace of chlorophyll at one point only, and on drying showed a bright yellow colour and weighed 0·165 grm. The water contained no perceptible formaldehyde, and, although it gave no distinct reduction with Fehling's test, left a residue of 0·052 grm. In spite of its long exposure, therefore, in the presence of an excess of CO_2 dissolved in water, the chlorophyll combined with carbon dioxide forming xanthophyll and a colourless, waxy residue dissolving readily in petrol ether.

Water alone has no effect on chlorophyll. Thus, films kept for one week in darkness in water deprived of all carbon dioxide were still quite green and apparently unaltered. On keeping the water saturated with carbon dioxide, the films after two weeks' darkness became dull yellow, and gave a pure yellow solution of xanthophyll soluble in cold alcohol, and leaving a colourless, waxy skin, soluble in petrol ether. This waxy material in all cases prevents the xanthophyll from dissolving in the water, which remains colourless, but when the xanthophyll is isolated it has the same properties as that obtained directly in chlorophyll extraction.

If oxygen is present as well as carbon dioxide, the water in the beaker

* This nitrogen may have come from the chlorophyll, but more probably remained dissolved in the water when the tube was sealed.

may show a faint trace of formaldehyde, but no reduction with Fehling's test.* Apparently, in darkness, a feeble oxidation of xanthophyll may take place as well as of chlorophyll. Chlorophyll, therefore, combines with carbon dioxide when present in a saturated watery solution in darkness, forming xanthophyll and a colourless waxy solid. In light, the same action takes place more rapidly and readily, and even under negative pressure. The rapid yellowing of grass leaves when cut fresh and heaped in masses is due to this action of carbon dioxide on chlorophyll. If a quantity of grass leaves is boiled and kept in a dense mass, in a few hours the *post-mortem* production of carbon dioxide has destroyed nearly all the chlorophyll, and large amounts of xanthophyll can be extracted, but very little chlorophyll.

Xanthophyll, Carotin, and Carbon Dioxide.

Experiments were made by exposing (*a*) dry films of xanthophyll and carotin in dry carbon dioxide, (*b*) watery solutions of xanthophyll, and watery emulsions of carotin in tubes sealed after saturating and filling with carbon dioxide. After a week's exposure the colour was unaltered, no evidence of combination could be seen, no new products appeared, and the enclosed gas consisted of pure carbon dioxide with occasionally a trace of nitrogen

General Conclusions.

The foregoing results indicate that the assimilation of carbon dioxide is not a simple process, as represented by the equation $CO_2 + H_2O = CH_2O + O_2$, in which chlorophyll merely absorbs the energy required, but is a very complex one, in which two pigments at least and their derivatives take part, and in which the equilibrium between the products and reacting substances determines the direction in which the reactions may take place, while light influences this equilibrium and strongly accelerates the tendency to oxidation on the part of all the pigments concerned.

According to Willstätter, amorphous chlorophyll is the methylphytyl ester of the tricarboxylic acid, $C_{31}H_{29}N_4Mg(COOH)_3$, or chlorophyllic acid, and has the formula $C_{31}H_{29}N_4Mg\begin{cases} COOH \\ COOCH_3 \\ COOC_{20}H_{39} \end{cases}$.

The phytyl radicle can be displaced. Thus Willstätter and Stoll† found

* Although a strong solution of formaldehyde gives a reduction with Fehling's test just like a reducing sugar, with a weak solution the slight excess of alkali on warming converts the formaldehyde into methyl alcohol and sodium formate. Hence dilute solutions of formaldehyde give Schiff's test and other sensitive tests, but do not give Fehling's test.

† 'Annalen,' vol. 378, p. 18 (1910).

that chlorophyll in the presence of the enzyme chlorophyllase and of ethylic alcohol forms methyl ethyl chlorophyllide or crystalline chlorophyll and the alcohol of phytyl (phytol)

$$C_{31}H_{29}N_4Mg\begin{cases}COOH\\COOCH_3\\COOC_{20}H_{39}\end{cases} + C_2H_5OH = C_{31}H_{29}N_4Mg\begin{cases}COOH\\COOCH_3\\COOC_2H_5\end{cases} + C_{20}H_{39}OH.$$

The same enzyme appears also to be able to produce the synthesis of chlorophyll from phytol and methyl ethyl chlorophyllide.

In any case, the phytyl radicle of chlorophyll is easily displaced, and there appears to be some evidence that the chlorophyll may be re-formed from xanthophyll and the colourless waxy residues of bleached chlorophyll. Thus, if in the first separation of chlorophyll a slight excess of water is added and the liquid kept in darkness in a separating funnel for one or more days, at the top is impure petrol ether chlorophyll, on the surface of the watery layer is an emulsion containing chlorophyll, impurities, and solid carotin, well below this the liquid is yellow with a white turbidity (A) and at the bottom is a clear yellow liquid (B). If B is evaporated to dryness and digested with cold alcohol, it yields xanthophyll. If an equal volume of A is run off, no chlorophyll can be extracted from it by petrol ether, or detected in it by the spectroscope. On evaporating it (in very feeble light), when nearly all the alcohol has been driven off, green skins of chlorophyll form. On filtering, the liquid is brown but turbid and again forms fresh chlorophyll on evaporating. If filtered as often as any green pigment separates, the ultimate dry residue may contain little or no xanthophyll as compared with the residue from B. The amount of chlorophyll recovered in this way may be sufficient to tinge the original volume quite green, and to mask the yellow colour when dissolved in alcohol and added to a fresh portion of the original liquid.

A large number of attempts were made to obtain chlorophyll from the colourless products of its oxidation and xanthophyll or carotin. In one experiment freshly bleached chlorophyll dissolved in hot alcohol was poured into water, the alcohol partially evaporated, and a watery solution of xanthophyll added. A little zinc dust was added, and the tube was sealed so as to enclose half its volume of air, so that on exposure to diffuse daylight oxidation took place at the surface, reduction at the bottom. After three days, on opening, partially evaporating, adding alcohol, filtering, and then adding first petrol ether and then water, the petrol ether showed a distinct green colour and red fluorescence and the spectrum of chlorophyll, although no chlorophyll was present originally. This result was, however, quite capricious. Out of some 20 experiments about three in four were

failures, although apparently repeated in exactly the same way. Possibly the stage of bleaching may be of importance, but in any case these results cannot be taken as establishing a fact but rather as suggesting a possibility, namely, that a reconstruction of chlorophyll may be possible from the products of its oxidation and from xanthophyll. There is no doubt, however, as to xanthophyll being one of the first decomposition products of chlorophyll, especially in the presence of CO_2 and sunlight. Although not claiming any exact quantitative accuracy for the following equations, they may express the probable process of carbon dioxide assimilation in the plant.

Stage 1.—Carbon dioxide and water combine with the phytyl base of chlorophyll, forming xanthophyll and oxygen. This will take place slowly in darkness but is accelerated by light.

$$4COOC_{20}H_{39}^{*} + 76CO_2 + 34H_2O = 4C_{40}H_{56}O_2 + 93O_2.$$

Stage 2.—A portion of this oxygen is used in the oxidation of the xanthophyll into phytyl, hexose sugars and formaldehyde, and the remainder is excreted from the chloroplastid. This takes place in light only.

$$4C_{40}H_{56}O_2 + 42H_2O + 17O_2 = 4COOC_{20}H_{39} + 8C_6H_{12}O_6 + 28CH_2O.$$

Stage 3.—The phytyl retakes its place in the chlorophyll molecule, the oxygen remaining ($76O_2$), which is equal in volume to the carbon dioxide absorbed, is exhaled, and the surplus formaldehyde is polymerised to hexoses, either by contact with the chlorophyll or with dilute alkali in the protoplasm around the chloroplastid.

The one point which cannot so far be repeated outside the living chloroplastid is the evolution of oxygen. Possibly with extracted chlorophyll, any oxygen set free in its substance at once combines with it. It is possible that in the living plastid some special separation is effected, or the magnesium of the chlorophyll may reduce some of the xanthophyll to the very readily oxidisable carotin, which might protect the chlorophyll from oxidation. Further, carotin and chlorophyll, being insoluble in water, oxidise much less rapidly when suspended in water than does a solution of xanthophyll. This gives a further possibility for the separation of oxygen at the surface of the chloroplastid. The separation of oxygen can hardly be produced by the action of a reductase reducing xanthophyll to carotin, for this would only give one of oxygen for every 19 of CO_2, and in addition, when magnesium (or zinc) reduces xanthophyll to carotin, no oxygen is set free.

The exact means by which the oxygen formed escapes without wholly

* For simplicity the rest of the chlorophyll molecule is omitted from the equation.

combining with the readily oxidisable chlorophyll needs further investigations, but the views put forward seem to give a reasonable temporary working hypothesis of the function of chlorophyll, xanthophyll, and carotin in the assimilation of carbon dioxide. A continuous liberation of oxygen would only be possible when stages 2 and 3 were carried out. The chemical reactions involved form a series of chemical changes which in part at least are reversible, and whose continuance in a definite direction is determined by light and by a continued supply of carbon dioxide at one end of the equation and by a continued removal of the sugar, formaldehyde, and surplus oxygen at the other end of the equation.

Summary.

No peroxides, organic or inorganic, are produced during the photo-oxidation of chlorophyll, xanthophyll, or carotin, but these substances, when exposed to light in the presence of an abundant supply of oxygen, may act as oxidases not only to themselves but also to substances with which they may be in contact, such as hydriodic acid, litmus, or guaiacum. Hence arises the "iodoxidase" reaction of bleaching chlorophyll, carotin, xanthophyll, and other pigments oxidising in light.

Chlorophyll and xanthophyll decompose during photo-oxidation into (*a*) solids and (*b*) a gas. The solids consist of colourless waxy substances and hexose sugars. The waxy solids are relatively small in amount in the case of xanthophyll. The gas is formaldehyde gas. With dry films in dry air free from carbon dioxide, relatively more formaldehyde is produced and less sugar, and the bleached residue weighs much less than the original solid. In moist air more sugar is formed and the residue may weigh nearly as much as the original solid.

Carotin oxidises more rapidly than xanthophyll or chlorophyll, and yields a little formaldehyde and a large amount of a colourless waxy solid, which may be a form of phytyl or of phytosterin.

Carbon dioxide combines with chlorophyll forming xanthophyll and a colourless waxy solid. The combination only takes place actively in the presence of water, and is accelerated by sunlight. Portion of the oxygen liberated by this reaction may oxidise the xanthophyll in the presence of sunlight to formaldehyde, sugar and phytyl, the latter retaking its place in the tricarboxylic chlorophyll grouping. No oxygen is set free when extracted chlorophyll is used. Some special means must exist in the chloroplastid of liberating the remaining oxygen without its oxidising the chlorophyll. Carotin may aid in protecting chlorophyll from photo-oxidation, and the reductase action of magnesium may be of importance.

There is some evidence suggesting the possibility that chlorophyll may be built up not only from ethyl chlorophyllide and phytyl alcohol, but also from xanthophyll and the products of the photo-oxidation of chlorophyll. The assimilation of carbon dioxide involves a complex series of chemical changes which are reversible in part at least, in which chlorophyll and xanthophyll play a direct chemical part, and in which light acts as an accelerating and possibly as a directive agency.

On Forms of Growth Resembling Living Organisms and their Products Slowly Deposited from Metastable Solutions of Inorganic Colloids.

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[PLATE 1.]

Graham, in his classical papers on colloids, draws attention to the remarkable dynamic properties possessed by matter in the colloidal form, whether as a hydrosol or a hydrogel. He states that "another and eminently characteristic quality of colloids is their mutability. Their existence is a continued metastasis. A colloid may be compared in this respect to water, while existing liquid at a temperature under its usual freezing point, or to a supersaturated saline solution. The colloidal is, in fact, a dynamical state of matter; the crystalloidal being the statical condition. The colloid possesses energia. It may be looked upon as the probable primary source of the force appearing in the phenomena of vitality. To the gradual manner in which colloidal changes take place (for they always demand time as an element), may the characteristic protraction of chemico-organic changes also be referred."*

It is only within recent years that the importance of these slow metastable variations in colloids so closely simulating the changes in living organisms, which are themselves metastable colloidal complexes, have been appreciated by a few authors, as thus clearly expressed by Graham over 50 years ago.

A metastable colloidal solution is, as Graham states, comparable to a

* 'Phil. Trans.,' vol. 151, pp. 183–224 (1861).

supercooled solution of a crystalloid, but it differs in that the appearance of the solid or "gel" phase of the colloid does not lead, as in the case of the crystalloidal state, to almost instantaneous separation of the excess of salt and establishment of a stable equilibrium, but instead of that, to a slow drifting towards an equilibrium which may go on for days or weeks or months, and vary in speed, or be actually reversed, with changing characters of environment.

It has been demonstrated by the work of Moore and Roaf* on the effects of variations in temperature upon the osmotic pressure of gelatine solutions, that a similar metastable condition arises long before any precipitation or passage from a hydrosol to a hydrogel occurs. If a mobile solution of gelatine at a temperature high above the point of gel-formation be raised a few degrees in temperature, the osmotic pressure is considerably increased in excess of the amount demanded by the gas law, showing that there occurs some dissociation in the solution-aggregate of the colloid. If, now, the temperature be allowed to fall back to the original point, only a small drop in osmotic pressure occurs at first and it requires some days before the original level is reached. This peculiar hysteresis requiring a prolonged interval of time for the passage from one condition to another is of great biological interest and may lie at the root of those cyclic alternations, of varying times in different tissues, so characteristic of living matter.

The present communication is concerned with another interesting similarity between inorganic colloids and living structures, namely, that the forms assumed as a result of these slow metastable depositions, or growths, so closely resemble lowly living organisms as to be, in many cases, most difficultly distinguishable from them. In our view, it is this close mimicry between colloidal deposits and living organisms which is responsible for more than one previous observer having described as living organisms such slow growths in metastable solutions.

Rapid osmotic growths between strong solutions of colloids and of crystalloids, capable in most cases of slowly precipitating one another and so forming precipitation membranes, have been studied in most painstaking and ingenious ways by a great host of observers.† These experimentalists have shown that similar effects to production of cell-membranes, skeletons, shells, and tests, and also of mitotic nuclear-division figures, and many

* 'Biochemical Journ.,' vol. 2, p. 34 (1907).

† Such as Gustav Rose, Runge, Böttger, Traube, Harting, Monnier and Vogt, Quincke, Leduc, Benedikt, Dubois, Herrera, Kuckuck, Albert and Alexandre Mary, and others. Accounts of the literature of the subject are to be found in Leduc, 'Mechanism of Life,' Rebman, London, 1911, and Quincke, 'Annalen der Physik,' 4te Folge, vol. 7, p. 631 (1902).

symmetrical and beautiful forms resembling those of lower aquatic animals may be reproduced by the action of such osmotic energy forms.

The experiments to be recorded in this paper follow much more closely, however, the lines of those devised by Dr. Charlton Bastian,* and there is an essential difference between the two types.

The forms obtained by the other observers depend upon diffusion effects between highly different solutions placed in close juxtaposition, and so producing steep gradients of variation of concentration with attendant rapid osmotic pressure changes. One of the two solutions is usually a hydrosol as in the Quincke and Leduc experiments, and the other is a fairly concentrated crystalloidal solution or a solid mass of crystals, which reacts with this, producing a precipitation membrane across which the diffusive actions occur. Fluctuations in deposition of this membrane and accidental variations in its thickness and resistance at various points, mainly account for the wonderful forms obtained. Here, doubtless, a great deal of the variations in effect are due to the rate of formation of the hydrogel and its subsequent alterations in properties after formation as degree of aggregation changes.

But in Bastian's type of experiment, there is no formation of a membrane, and no osmotic pressure or diffusion velocity effects arise. The two solutions are thoroughly mixed up from the outset, but in such proportions that the system is just metastable, and so that a deposit is very slowly formed. As will be pointed out later, in describing the details of making up the colloidal solutions, these are just the conditions reached by following the instructions given by Dr. Bastian.

At the outset it is desirable to state that it is our intention to deal only with the peculiar and interesting forms in which growths appear in such metastable solutions of inorganic colloids, and to leave for the moment on one side the larger question as to whether actual living organisms appear in them. The growths we have observed increase in many cases when left in ringed solutions for some days between slide and coverslip. But we may say that we have not been able to obtain experimental evidence that they contain organic carbon compounds, and have not been able to sub-culture them in other media, as has been claimed by Dr. Bastian in regard to his experiments.

The deposits or growths stain with dyes, such as methylene blue, but this, in our opinion, is not evidence that they are organic, for inorganic colloids also adsorb dyestuffs readily and give a staining effect. The growths we

* See 'The Origin of Life,' by H. Charlton Bastian, M.D., F.R.S., Watts & Co., London, 1911.

have had under examination certainly do not contain cellulose, for they give a negative reply to the iodine and sulphuric acid test for cellulose.

It is now established that many colloidal inorganic substances in presence of sunlight and of water and carbon dioxide can synthesise organic bodies,* so there is no inherent impossibility that living organisms containing organic carbon compounds can arise from inorganic matter when proper conditions of environment, energy supply, and time are satisfied. But we have been unable to observe anything which we could describe as a living organism arise from these inorganic colloids.

The forms which arise in metastable solutions of inorganic colloids are worthy of consideration as illustrating a mechanism by means of which, when the steps of evolution of the organic from the inorganic have become understood, the study of the origin of the morphology of the microscopic forms of life can find a basis.

At the present moment, and with the lacunæ now existing in our knowledge of the stages intermediate between inorganic evolution and organic evolution, even did undoubted living organisms arise in sterilised and hermetically sealed tubes, their origin would be looked upon with suspicion and ascribed to hypothetical unkilled germs or some chance contamination. The intermediate ground which must be cleared is that of the morphology of inorganic colloids, and the properties of these as catalysts enabling them to build up and synthesise organic compounds. It is from this point of view that we submit a preliminary study of the forms of growths originating in metastable solutions of inorganic colloidal solutions.

The glass tubes used in our experiments were of the same type as those employed by Dr. Bastian in his experiments, and were manufactured for us by the same firm. These tubes are made from glass tubing about 3 cm. in diameter. A rounded bottom of the same diameter as the tube is first blown, then at about 7 cm. from the bottom the tube is drawn off to a tapering point and sealed at the end, the whole length of the tube, including both wide and narrow parts, being about 17 cm.

The tubes were delivered to us sealed, and were first broken when the experiment was to be commenced by filling in the colloidal solutions. It is quite obvious that in the process of manufacture the whole internal surface of the tube would require to be so strongly heated by the glass-blower as to incinerate and destroy utterly any possible organic fibres from entrance of dust or vegetable fibre, and the greatest care was taken by us to prevent ingress of adventitious fibres in any of the subsequent observations and the after manipulations for microscopic observation. All slides and coverslips

* Moore and Webster, 'Roy. Soc. Proc.,' B, vol. 87, p. 163 (1913).

were carefully cleansed with chromic acid and alcohol, and were flamed and examined with the microscope before using them. All pipettes and lifters were also flamed. In the manufacture of the tubes the formation of the rounded bottom of the tube must have heated the glass red-hot for a distance of 3 or 4 cm. at least from the bottom, and the drawing out at the top must similarly have heated all the remainder. We are accordingly quite satisfied that the deposits or growths we are dealing with were actually formed in the tube-contents after the tubes were sealed off and left standing.

Each tube after filling was hermetically sealed and then sterilised by heating in a steam autoclave for 15 minutes at 110° C. The tubes have a capacity of about 100 c.c. and were about one-third filled with the selected mixtures of colloids. The filling was carried out by nipping off at the narrow end, warming the tube, and dipping into the prepared mixture of colloids contained in another sterilised tube (as in the process of filling a thermometer bulb); the tube was then resealed and autoclaved as stated above.

The solutions used were those described by Dr. Bastian as the "yellow" solution and the "colourless" solution, and the same solutions were also employed with the addition of two drops of 5-per-cent. sodium carbonate solution in 30 c.c. of total fluid. This small amount of sodium carbonate was added in order to provide material for formation of organic carbon compounds if there was any tendency towards such a growth of organic matter. In no case, however, have we been able to assure ourselves that there was any formation of organic carbon compounds. We feel certain that there was no appreciable growth of such compounds, but would like to leave this question open because it is so difficult of solution.

At the expiration of nearly seven months some of the tubes were opened and examined microscopically, when the growths shown by the microphotographs (Plate 1) were observed.

Some of the growths observed closely resemble exceedingly fine vegetable fibres, such as filaments of cotton fibre, but finer in structure. Some are rounded like silk fibres, others show flat bands like cotton fibres. Other growths show branched and sometimes transversely divided filaments like hyphæ of moulds. These inorganic growths, indeed, so closely resemble vegetable fibres that we were assured by two competent histologists, to whom we showed them, that they were fibres obtained by leaving cotton filaments on the slides in the process of preparation, and that the use of the reagents for cellulose would certainly demonstrate this fact.

This suggestion was valuable because it showed quite clearly, on application, that the growths were not organic, or, at least, were not cellulose; for

these peculiar deposits do not stain when treated with iodine and sulphuric acid. It is, however, exceedingly difficult, in the technique of microscopic examination, to exclude contamination from the air, and also in some cases the nature of cellulose fibres might be so altered that they no longer gave the typical reactions of cellulose.

Experimental Procedure.

The solutions used were made up following as closely as we could the procedure recommended by Dr. Bastian, and in order to do so, we obtained the pharmaceutical solutions from the same manufacturing chemists. The "liquor ferri pernitratis" used was obtained from Messrs. Martindale, and the "sodium silicate" (sp. gr. 1·44) from Messrs. Allen and Hanbury. Our thanks are due to Dr. Bastian for aiding us in obtaining the same materials, as nearly as possible, which were used for his own experiments. The sodium silicate solution was diluted before use with an equal volume of distilled water as recommended by Dr. Bastian.

"Liquor ferri pernitratis" of the British Pharmacopœia contains 3·3 per cent. of iron, and of this 8 drops, from a dropper giving 29 drops to the cubic centimetre, were added to a total volume of 30 c.c. of distilled water, and a minute amount of sodium silicate, viz., two drops of the sodium silicate (Martindale) diluted one half. The percentage of iron contained in the colloidal solution in which the growths or deposits appeared would accordingly be approximately 0·03 per cent. The amount of sodium silicate is also very minute, and is just sufficient, when instructions are followed, to send both itself and the ferric nitrate into the metastable colloidal condition.

The instructions of Dr. Bastian are to use constantly 8 drops of the iron salt solution to the ounce (about 28 c.c.) of distilled water, and then, according to the varying alkalinity of the sodium silicate solution, to add 2, 3, or 4 drops of it, so as to obtain a mixed solution that is faintly acid or neutral, and which on boiling for 10 minutes yields, after standing for some time, only a very small amount of deposit.' Solutions giving no deposits after boiling, or those which, on the other hand, are completely deposited, form no growths. This balance was obtained, in the case of our solutions, when 8 drops of the ferric nitrate solution and 2 drops of the sodium silicate solution were used to 30 c.c. of distilled water; the equilibrium is a very sensitive one.

Now it is to be observed here, that the ferric nitrate is an acid solution readily thrown down by alkali, and the sodium silicate is an alkaline solution from which silicic acid is readily thrown out by acid. When these two solutions are mixed in properly balanced quantities, as is done

in these experiments, there is obtained a mixture in common solution of two metastable colloids, viz., ferric oxide and silicic oxide. These are the favourable conditions for the deposit of the peculiar growths that have been observed.

The above solution, following Dr. Bastian, is called the "yellow" solution. The colourless solution, which probably contained one metastable colloid only, viz., silicic acid, was prepared, following again fairly closely Dr. Bastian's directions. To 30 c.c. of distilled water 2 drops of the sodium silicate solution were added, then 6 drops of "dilute phosphoric acid solution, B.P." (Martindale), and a few crystals of ammonium phosphate (Martindale).

Here, again, an alkaline silicate solution is taken which, with the phosphoric acid solution, would yield at first a metastable colloidal solution of silicic acid, and then with a greater excess a precipitate of silicic acid. The alkaline and acid salts are just so balanced in the above proportions that only a small proportion of the silicic acid is thrown out on autoclaving, or within a short period of a few hours thereafter.

Attention is drawn here to these important points in the chemistry of inorganic colloids, because Dr. Bastian by long and patient experimentation appears to have arrived at the proportions most favourable to the appearance of these growths. To the physical chemist it is obvious that the proportions are just those which will give rise to a metastable solution of a colloid, or a metastable mixture of colloids.

It is under such conditions that slow deposition will take place and cyclic variations can occur, if there be, during a long time interval, up-and-down variations in physical factors of environment. Such are also the conditions in a living cell, and hence the interest in the fact that so many of the appearances shown by the growths are those seen in cell-products.

In October, 1912, between the 18th and 24th, 48 tubes were prepared and autoclaved as above described, the contents of the sets being as shown in the following Table; the qualities of reagent stated were added to 30 c.c. of distilled water in each case.

Thus there were a dozen tubes of each class, viz. one dozen "yellow" solution, one dozen "colourless" solution, one dozen "yellow" solution *plus* sodium carbonate, and one dozen "colourless" solution *plus* sodium carbonate.

It may be said at once that the subsequent examination at a period of seven months afterwards showed no observable difference in the growths, either favouring or deterrent, due to the sodium carbonate.

Preparation of Tubes.

No. of tube.	Contents "yellow."	No. of tube.	Contents "colourless."
4	2 drops silicate, 8 drops ferric nitrate, 2 drops sodium carbonate.	*4a*	2 drops silicate, 6 drops phosphoric acid, ammonium phosphate, 2 drops sodium carbonate.
5	„ „ „	*5a*	„ „ „
6	„ „ „	*6a*	„ „ „
October 18, 1912. The 6 tubes were autoclaved for 15 minutes at 115° C. For a few *seconds during the period the temperature reached* 120° C.			
7	As above, except no sodium carbonate was added.	*7a*	As above, with omission of sodium carbonate.
8	„ „ „	*8a*	„ „ „
9	„ „ „	*9a*	„ „ „
All heated for 15 minutes in autoclave, temperature 105–115° C., on October 21, 1912.			
10	Same as Nos. 7, 8, and 9. No sodium carbonate.	*10a*	As Nos. *7a*, *8a*, and *9a*. No sodium carbonate.
11	„ „ „	*11a*	„ „ „
12	„ „ „	*12a*	„ „ „
13	„ „ „	*13a*	„ „ „
14	„ „ „	*14a*	„ „ „
15	„ „ „	*15a*	„ „ „
All 12 tubes autoclaved for 15 minutes at 108–110° C. on October 22, 1912.			
16	As Nos. 4, 5, and 6. Contain sodium carbonate.	*16a*	As Nos. 4, 5, and 6. Contain sodium carbonate.
17	„ „ „	*17a*	„ „ „
18	„ „ „	*18a*	„ „ „
19	„ „ „	*19a*	„ „ „
20	„ „ „	*20a*	„ „ „
21	„ „ „	*21a*	„ „ „
Heated in autoclave for 15 minutes at 110° C. on October 23, 1912.			
22	As Nos. 7, 8, and 9. No sodium carbonate.	*22a*	As Nos. 7, 8, and 9. No sodium carbonate.
23	„ „ „	*23a*	„ „ „
24	„ „ „	*24a*	„ „ „
Heated in autoclave for 15 minutes to 112° C. on October 24, 1912.			
25	As Nos. 4, 5, and 6. Contain sodium carbonate.	*25a*	As Nos. 4, 5, and 6. Contain sodium carbonate.
26	„ „ „	*26a*	„ „ „
27	„ „ „	*27a*	„ „ „
Heated in autoclave for 15 minutes on October 24, 1912.			

On October 29, 1912, the four dozen tubes were disposed as follows:—

West window.	East window.	North window.	South window.	In dark cupboard.	
Nos. 10, *10a*	Nos. 12, *12a*	Nos. 5, *5a*	Nos. 19, *19a*	Nos. 4, *4a*	Nos. 21, *21a*
„ 11, *11a*	„ 22, *22a*	„ 6, *6a*	„ 20, *20a*	„ 18, *18a*	„ 25, *25a*
„ 16, *16a*	„ 26, *26a*	„ 7, *7a*	„ 14, *14a*	„ 9, *9a*	„ 23, *23a*
„ 17, *17a*	„ 27, *27a*	„ 8, *8a*	„ 15, *15a*	„ 13, *13a*	„ 24, *24a*

The examination of the contents of the tubes and the deposits in them under the microscope were commenced on May 29, 1913, that is about seven months after they had been filled and sealed and sterilised.

The peculiar growths shown in some of the appended microphotographs were then seen in all the tubes examined, more abundantly in the "yellow" solution tubes than in those containing "colourless" solution, but still plentiful in these also. The tubes in the windows were richer in growth than those kept in darkness, but the latter did contain growths also, and it would be impossible without more evidence to say whether the greater result in the windows might not be due to greater diurnal fluctuations of temperature than those that take place in the dark cupboard.

The chief appearances observed in the contents of these tubes are:—(1) Patches of sometimes fine, sometimes coarse, granular deposits with fine fibres running in them. (2) Chains of dots, sometimes slightly elongated, like micrococci or short bacteria. (3) Branching coarse fibres like hyphæ of a fungus. (4) Coarse fibres, sometimes rounded, sometimes flat and twisted like a cotton fibre; these are very long, and sometimes run more than the whole diameter of a low-power field, sinuous on their course and quite unlike anything crystalline; as mentioned earlier, these may be adventitious. (5) Excessively fine fibrils, also very long and often taking the most fantastic shapes, sometimes they form a network like a fibrin network, at other times they are single and convoluted into the most intricate knots or loops; this type of fibre is finer than any cotton or silk fibre.

All the tubes were examined carefully, but it would serve no purpose to write a detailed description of each, as the appearances were so similar; so the following descriptions may serve as examples:—

Tube No. 11 (West window, Yellow solution, no soda). Examined May 29, 1913.—This shows plenty of both fine fibres and masses of débris, and small black dots, sometimes in rows like cocci. A very good dip is obtained from supernatant fluid, near wall of tube, away altogether from the coarse deposits, showing very fine branching and interlacing fibres, like minute hyphæ.

Tube No. 17 (West window, Yellow solution, plus soda). Examined May 29, 1913.—This is very similar to the above and contains both types of fibres.

Tube No. 26 (East window, Yellow solution, plus soda). Examined May 30, 1913.—Very rich, both in fine, interlacing fibres and in the long, coarse, and twisted fibres. The latter do not give any cellulose reaction with iodine and sulphuric acid. Also, they are yellow hued and evidently contain colloidal iron. Other slides examined give the same results. Many long, delicate fibres often twisted into intricate loop patterns. Only yellow in colour; no iodine staining. The sulphuric acid appears to dissolve out much of the

granular matter resembling protoplasmic débris, and leaves the long fibres and shorter nests of exquisitely fine fibrils more clear.

In the appended microphotographs are shown growths from these and other tubes of the series.

These growths, obtained in hermetically sealed tubes, autoclaved up to 110–116° C. after sealing, and in tubes in which all organic matter must have been completely destroyed in the process of manufacture, appear to us to be proven conclusively not to be caused by any contamination with adventitious organisms. Whatever view may be taken as to the nature of these appearances, our opinion is that they are not adventitious or due to contamination, but that they arise *de novo* in the tubes by a process of growth or deposition from the balanced colloids.

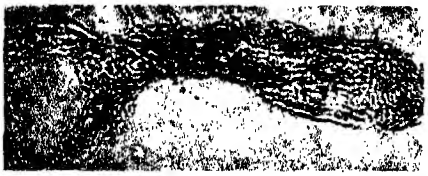
While the boiling or autoclaving was essential in the first stages of the investigation in order to rule out contamination, this process undoubtedly disturbs to a great extent the metastable condition of the colloids and throws out a good deal of the substances, and, in addition, probably interferes with the labile condition of the remainder short of actually precipitating it.

In order to get rid of this inactivation by heat, it was determined, after the growth in autoclaved solutions had once been settled, to experiment with tubes containing metastable colloidal systems which had only been exposed to a moderate degree of heating so as not to disturb any thermo-labile colloids present. The result was that much more growth occurred of all the constituents described.

A series of tubes, filled as described above, were prepared in April, 1914; but, instead of autoclaving, these tubes were heated for 20 minutes to 50° C. and then allowed to stand for two months. At the end of the period, this set of tubes showed very little deposit, but the forms found in the deposit were beautifully developed.

It is to be remembered here that none of the tubes contained any nutrient materials for ordinary micro-organisms, and that heating to 50° C. for 20 minutes would have destroyed any common forms likely to be present. The forms, moreover, closely resembled those found much more sparsely in the autoclaved tubes, and the explanation of the abundance appears to lie in the fact that the colloids were left in a more labile condition.

It is admittedly impossible to exclude completely the objection of contamination in this latter series of experiments. Other methods of causing metastability in mixtures of inorganic colloids and so producing growths more rapidly have been studied and are described in the succeeding paper.



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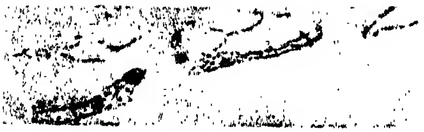

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DESCRIPTION OF PLATE.

Fig. 1.—Deposit from Tube No. 8, (×250.) After autoclaving for 15 minutes at 105–115° C.

Fig. 2.—Deposit from Tube No. 4. (×250.) Autoclaved previously, 105–115° C.

Fig. 3.—Symmetrically Arranged Deposit from Tube No. 7. (×250.) Not really crystalline. Autoclaved.

Fig. 4.—Coarse Feathery Deposit from Tube No. 17. (×250.) Autoclaved.

Fig. 5.—Fine Deposit from Tube No. 5. (×250.) Autoclaved.

Fig. 6.—Deposit from Tube No. 7. (×250.) Autoclaved.

Fig. 7.—Hyphæ-like Deposit from Tube No. 20. (×240.) Autoclaved.

Fig. 8.—Fine Deposit, Tube 20. (×240.) Autoclaved.

Fig. 9.—Long Looped Fibre from "Colourless" Solution. Metastable silica only. (×440.) Autoclaved.

Fig. 10.—Mixed Deposit in "Yellow" Solution. Metastable silica and ferric hydrate. (×240.) Autoclaved.

Fig. 11.—Hyphæ-like Deposit in Yellow Solution. (×240.) Autoclaved.

The Production of Growths or Deposits in Metastable Inorganic Hydrosols.

By Prof. BENJAMIN MOORE, M.A., D.Sc., F.R.S.

(Received February 6, 1915.)

[PLATES 2 AND 3.]

The results described in the preceding paper conducted me to the study of other methods for obtaining these growths.

The fundamental law established by Pasteur, and now universally confirmed, that organic growth cannot occur in sterilised organic media, leaves a curious hiatus between inorganic evolution and organic evolution.

It is a remarkable historical fact that organic evolution was firmly established a full generation before inorganic evolution, and that, with the exception of certain ingenious hypotheses, the theory and facts of inorganic evolution have only been partially ascertained in late years.

The problem presents two distinct yet closely related lines of attack. One concerns the method by which organic compounds can be built up from inorganic sources, and is more purely a question of energy-transformation; the other is related to the morphology, or minute anatomy, in the region lying between the inorganic and the organic, and deals with the colloidal inorganic forms preceding the organic structures. Energy-transformations, although of

a different type, underlie the morphology also, but the methods of research vary in the two cases.

It is necessary to study how the organic constituents of living organisms with their high content of energy may be formed by inorganic catalysts by utilising other types of energy, such as sunlight; it is also equally important to pay attention to those visible microscopic forms which colloids assume closely resembling the lower living forms, as the region between inorganic and organic is traversed.

It is highly important to be accurate and rigid in such observations, and to realise that they are distinct from the discoveries of Pasteur. It is proven that living organisms are least likely of all to arise in sterilised organic media, but that has nothing to say as to how living organisms first arose, or arise to-day.

The problem is *this—What is the link between the organic and the inorganic?*

The forms of growth of crystals have been studied and classified, and it seems reasonable that similar attention should be paid to the forms in which colloids present themselves.

The study of the more complex forms of growth lying between crystals and living organic forms is closely beset with difficulties, on account of the very difficulty of excluding living forms, for one is working here upon the stage which is nearest to acknowledged living types.

The effort in the present research had accordingly been to exclude as far as possible any contamination, and to devise methods of research which would yield only inorganic forms under conditions exclusive of life.

The objective designedly was that of obtaining colloidal growths, and observing how closely the appearances approach those of the lowliest known forms of living organism.

In such a quest, time is an element of the utmost importance. The nature of the colloidal solutions is such that the usual crude procedures of sterilisation are difficult or impossible of application. Antiseptics cannot be employed, because they precipitate the colloid. Heating in an autoclave above the normal boiling point of water, at atmospheric pressure, to 110 to 115° C., often throws the complex inorganic colloid out of solution and activity.

In order to rule out actual life processes events are hastened so as to obtain in a few minutes effects of the same type as those which are usually produced in days or weeks. It is not, therefore, to be expected that the same delicacy and degree of organisation will be obtained as in the slower processes of life, or of metastable colloidal solutions left to themselves for much longer periods.

The difference is similar to the well-known difference in crystallography of the slowly crystallising and the rapidly crystallising solution.

It was pointed out by Graham, as quoted in the previous paper, that time is an essential factor in all colloidal solutions, and that in this respect the colloids approach living organisms.

The first method attempted was that of using stronger solutions of the two reagents of the previous paper, and bringing these together in proportions as close to the point of actual precipitation as possible, so as to hasten operations of growth in order that contamination by organisms might be safely excluded.

For this purpose a 1-per-cent. solution of ferric nitrate and a 1-per-cent. solution of sodium silicate, each in distilled water, were prepared, and from the two a metastable colloidal system was constructed.

In mixing the two solutions the ferric nitrate solution must be taken as the basis and the silicate solution be slowly added to this, for if the reverse procedure be adopted the silicic acid is at once thrown completely out of solution.

If, however, a volume of the ferric nitrate solution be taken, and the sodium silicate solution be added to it drop by drop with constant shaking, it is found that, although every drop produces a precipitate, this, on shaking, redissolves, and no permanent precipitate occurs up to a certain point. Instead, a common solution is formed of colloidal ferric hydrate and colloidal silicic acid.

This point is reached when about 4 c.c. of the silicate solution has been added to 10 c.c. of the ferric nitrate solution. If this point be somewhat exceeded, a brownish-coloured precipitate appears permanently. On examination under the microscope this precipitate is seen to consist of fragments of membrane shrivelled and corrugated and showing thickened tortuous lines like embedded fibres in the substance of the membrane. Short pieces of fibrils project at places, at the borders of the shreds of membrane. The appearance suggests that fibrils are first thrown out of solution, and that subsequently in the meshes of the fibrils more connecting colloid in the gel form is deposited in a thin layer, so forming the membranous shreds of the precipitate.

This view is supported by the appearances shown when the metastable point is not passed. If 4 c.c. of the silicate solution be slowly added with continued agitation to 10 c.c. of the ferric nitrate solution, a slightly opalescent solution is obtained. This shows, on examination microscopically, very few membranes, but a large number of rather coarse fibres, branching and tortuous.

These are not so well developed as those more slowly grown in the

experiments of the preceding paper with the same reagents, but with care fairly well developed networks of fibres may be obtained by this method.

The next method employed was that of mixing two colloidal solutions of opposite cataphoresis (or electrical sign of the colloid), until the point of permanent precipitation was just being approached. The pair of colloids first tested consisted of colloidal ferric hydrate and colloidal silicic acid, each prepared by Graham's method. The two colloidal solutions used were freshly prepared and, when examined microscopically before admixture, were found to be free from fibrils.

In a series of test-tubes varying volumes of the two colloidal solutions were then mixed together, so as always to yield a constant total value of 10 c.c., and the mixture in each case was immediately shaken up and allowed to stand. Thus, in the experiment from which the microphotographs shown in Plate 2, fig. 1, were obtained, a series of tubes were prepared as follows:—Tube No. 1, 9 c.c. colloidal ferric hydrate plus 1 c.c. colloidal silicic acid; tube No. 2, 8 c.c. colloidal ferric hydrate plus 2 c.c. colloidal silicic acid; tube No. 3, 7 c.c. colloidal ferric hydrate plus 3 c.c. colloidal silicic acid, and so on. The colloidal ferric hydrate contained 0·136 per cent. of Fe_2O_3, and the silicic acid approximately 0·1 per cent. of SiO_2, and the above experimental procedure demonstrated that the best admixture for the rapid production of fibrous growths was 8 c.c. of the colloidal ferric hydrate solution to 2 c.c. of the colloidal silicic acid solution.

When mixed in these proportions and then in a few minutes examined under the microscope, magnificent networks are seen which are stained a pale yellow colour in the coarser fibres. Some of the fibres are so delicate and fine that they are only visible with a high magnification and using diminished illumination. Others, such as those shown in the microphotographs of fig. 1, are coarse and easily visible with the low power. Photography of these products is exceedingly difficult, and gives but a poor impression of the networks as seen under the microscope. Some of the medium-sized fibrils are double contoured, they branch, and in many cases show nodulation, cross striations, or divisions. In fact, many of the appearances presented by growing hyphæ are closely simulated.

It has not been possible for me to observe the mode of growth of these fibres. Large numbers are present almost at once when the two colloidal solutions are mixed, for the mixed solution examined straightway with the microscope shows them from the commencement. On careful examination of a freshly mixed preparation microscopically, with the greater part of the illumination cut off, and a magnification of about 400-500 diameters, exceedingly delicate networks and long branching fibrils may often be just

detected, and it would seem probable that the coarser fibres also arise in this way from at first almost ultramicroscopic rows of granules passing out of solution and joining up to form delicate fibrils. These delicate fibrils then thicken up and become more obvious. Looking at the most delicate fibrils actually visible, the eye so slowly takes up the details and one becomes so gradually conscious of the ramifications, that a deceitful impression is easily obtained, that the structures are actually growing under the eye. But on no occasion could I be certain that I had observed actual growth of a network.

A considerable number of observations were taken to determine whether there was any growth at the ends of the coarser fibres, but none could be detected. In many cases where slide and coverslip preparations were preserved for some weeks, there did, however, appear to be an increase in the growths, and appearances were observed of growths which certainly were characteristic and well developed, and had not been seen on earlier examinations of the same preparation. Often, with this pair of colloids, transition stages in the formation of membranes from fibres are to be seen, as is illustrated, for example, on the right-hand side of the lowest photograph in fig. 1. The appearances given by this method of mixing two opposite colloids are exquisitely beautiful and often intricate in design, although they are produced so rapidly.

Also it is important to stress the point that there can be here no contamination effect with adventitious living organisms. Two colloidal solutions are taken, one of a positive, the other of a negative colloid, each solution is examined carefully by the microscope and found to be clear and free from growths, the two are carefully mixed, and there, in a period of a few minutes, are the most delicate and intricately interlaced patterns of long and branching fibrils, as well as growths of coarse fibres.

The next pair of colloidal solutions investigated were ferric hydrate and "colloidal sulphur α" prepared as described by Quincke (*loc. cit.*). The colloidal sulphur α was obtained by taking distilled water previously sterilised by boiling and allowing it to cool. Two portions of the cooled sterile water were taken and saturated respectively with sulphur dioxide and hydrogen sulphide; then to 20 volumes of the sulphuretted hydrogen water was added one volume of the sulphurous acid solution. The result at once is a fine colloidal suspension, or solution, of sulphur particles. This shows microscopically a multitude of fine round granules all in rapid Brownian movement; on standing for about two days the particles show a tendency to grow together into fine coccal-like chains.

The solution may be diluted without precipitating and also may be boiled, it may even be autoclaved at 110° C. for a few minutes with only partial

precipitation, but prolonged autoclaving throws it out of solution. The amount of coagulum when the sulphur passes out of solution demonstrates that there is ultramicroscopic sulphur in colloidal solution in the fresh preparation, in addition to the particles mentioned above. A determination made by evaporating a known volume to dryness and weighing the sulphur gave 0·48 per cent. in the solution as prepared above.

This solution was diluted tenfold before use, as was also the colloidal ferric hydrate solution used for equilibration with it, so that the concentrations of the two solutions as actually mixed were approximately: colloidal sulphur α, 0·048 per cent.; colloidal ferric hydrate, 0·136 per cent.

In order to exclude entirely growths of "organic carbon" organisms, that is "living" organisms, both solutions, while still separate, were put into a steam autoclave at 110° C. for 10 minutes; this caused partial precipitation. The two solutions were then allowed to cool and when cold were mixed as in the following scheme:—

	Test-tube.								
Number	1.	2.	3.	4.	5.	6.	7	8.	9.
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
Ferric hydrate solution......	9	8	7	6	5	4	3	2	1
Sulphur α solution	1	2	3	4	5	6	7	8	9

There was no complete precipitation in any of the tubes, but a varying amount of increase in the opalescence.

Microscopic examination as rapidly as possible within a few minutes of making the admixtures showed growths in all, but most rapidly and abundantly in test-tubes Nos. 2 and 3. Microphotographs of the growths obtained when 8 c.c. of the colloidal ferric hydrate and 2 c.c. of the sulphur solution were mixed are shown in fig. 2. The growths here again, when sufficiently coarse, give the appearance by a yellowish colour of an iron salt.

The finest threads are only visible in dim illumination. The growths, again, in this case do not yield good microphotographs. All sizes of fibre are seen here from the finest fibrils up to quite coarse fibres, like structures from plants.

Spontaneous Growths in Colloidal Silicic Acid only.—It is well known from the classical experiments of Graham that sufficiently dialysed silicic acid solution is spontaneously metastable, and, after perhaps days, months, or years of keeping, passes out into a solid jelly. It is rather remarkable that

it has not before been examined microscopically at various periods during this metastable existence.

The actual setting into a thick jelly does not appear to yield microscopic structures, probably because this change at its onset is rapid, and so yields only ultramicroscopic structures. But the jelly, at first clear, gradually shows an increasing opacity after setting, and then, if broken up and examined under the microscope, shows flat membranous scales or plates like those described above for the more coarsely precipitated colloidal ferric hydrate.

The most interesting appearances, however, are those found before jelly formation in the perfectly mobile colloidal solution, when the solution is so dilute (1–2 per cent. of SiO_2) that it remains fluid for some weeks.

Since these growths form slowly, as great care as possible was taken, by autoclaving the mother-solutions of sodium silicate and hydrochloric acid and the dialysing apparatus and all glass apparatus and containers, to prevent infection adventitiously from without.

The growths obtained with colloidal silicic acid are illustrated by the microphotographs of figs. 3 and 4. No special care was taken in autoclaving the mother-solutions in the experiment shown in fig. 3, but it is to be remembered that these are strong hydrochloric acid and strong sodium silicate, neither of them suitable media for the growth of organic germs.

After mixing and dialysing, the colloidal silicic acid was run off into a stoppered bottle which had been sterilised by blowing live steam through it. The growths shown were observed after standing for about three weeks at laboratory temperature. The solution contained just over 2 per cent. of SiO_2, had been dialysed for 48 hours, and coagulated in about five weeks' time.

The coarser growths shown in fig. 3, A, B, C, and D, are obtained occasionally when a stronger solution (about 3 per cent.) of colloidal silicic acid is left for two or three days ringed round with gold size. They appear to form when slow massive formation of a gel phase occurs in a fairly strong solution accompanied possibly by slow concentration due to slight evaporation. These forms yield often very beautiful patterns with ramifying and branching fibres.

The coarser fibres seen in the micro-photographs are not cracks in a contracting jelly, but a more solid phase actually growing in a more fluid phase; this is well seen at the growing edge of such a tuft of fibres, as also from fine structural details in the individual fibres.

The appearances seen in fig. 3, E and F, and in all the photographs of

fig. 4, were obtained in a specially devised experiment in which everything for the obtaining of the colloidal silicic acid solution was sterilised beforehand, and then the whole experiment carried out under aseptic conditions.

The dialysis was made in a seamless test-tube of thick parchment paper, measuring approximately 5 cm. in diameter and 20 cm. in length. This dialysing tube at the outset was thoroughly boiled in distilled water, the distilled water used for diluting the solutions and the solutions themselves were also boiled, as also glassware and rubber cork of the apparatus now to be described.

The colloidal silicic acid solution itself when formed cannot be sterilised, as it is by such a process coagulated and thrown out of solution, but the ingredients from which it is made can be autoclaved.

The following disposition of the experiment was accordingly made. A rubber cork, pierced with three holes (through two of which the stems of two glass separating bulbs provided with glass taps passed), was taken and fitted over the mouth of the dialysing tube (5×20 cm.) described above. The dialysing tube was firmly fixed on the rubber cork with thread. Through the third hole in the rubber cork passed a glass tube, bent twice at right angles and narrowed to a fine point at its outer end. The inner end of this tube when in position passed to the bottom of the dialysing tube, and its outer end was either hermetically sealed or sealed with a mercury seal in a small test-tube surrounding its end. The purpose of this third tube was to draw off a sample of the dialysate daily into sterilised test-tubes.

Ten cubic centimetres of distilled water was placed in the dialysing tube. In one separating bulb were placed 20 c.c. of strong, pure hydrochloric acid and 10 c.c. of distilled water; in the other, 13 c.c. of a 38·5-per-cent. solution of sodium silicate and 27 c.c. of distilled water. The upper openings of the two separating bulbs were stoppered with cotton wool.

The whole apparatus, so prepared and filled with the solutions, was placed in a large autoclave and raised by steam to a temperature of 110° C. for a period of 15 minutes. The steam was then shut off and the whole allowed to cool. The apparatus was taken from the autoclave and, by opening the tap on the separator containing it, the hydrochloric acid was allowed to run into the dialysing tube. The sodium silicate solution in the other separator was then allowed to pass in, accompanied by constant shaking.

The dialyser so fitted up was immersed in a large beaker in a running stream of Liverpool tap water. It might be objected that sterilised distilled water ought to have been used here, but a properly sterilised and screened-off supply of cold sterilised distilled water is an exceedingly difficult matter to arrange. So it was determined to rely upon the impermeable properties

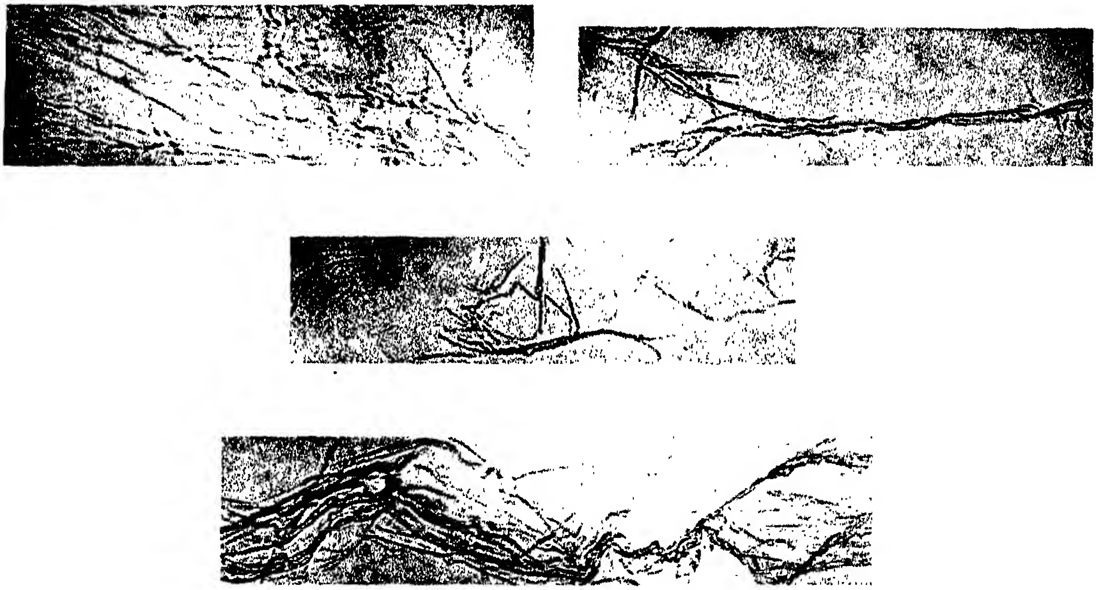

Fig. 1.

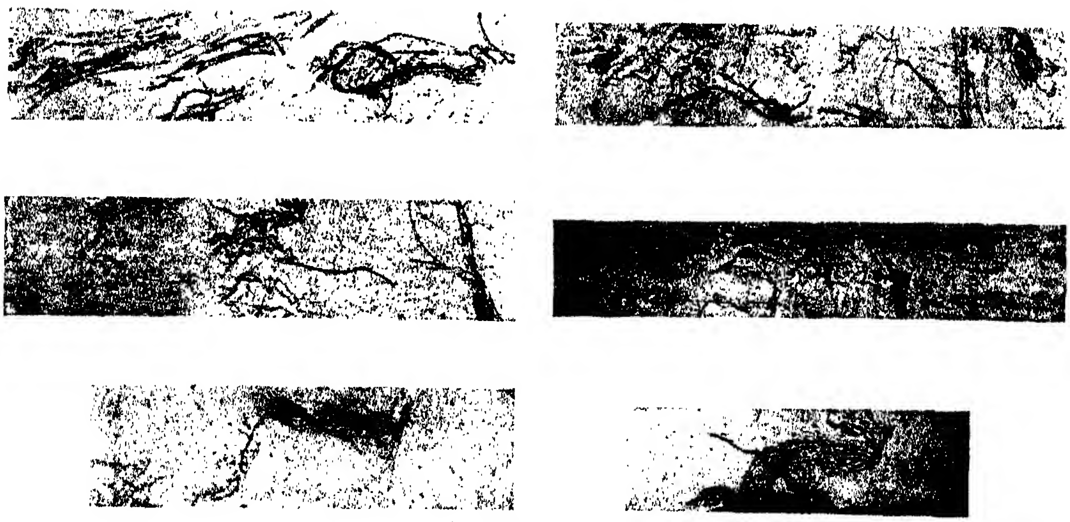

Fig. 2.

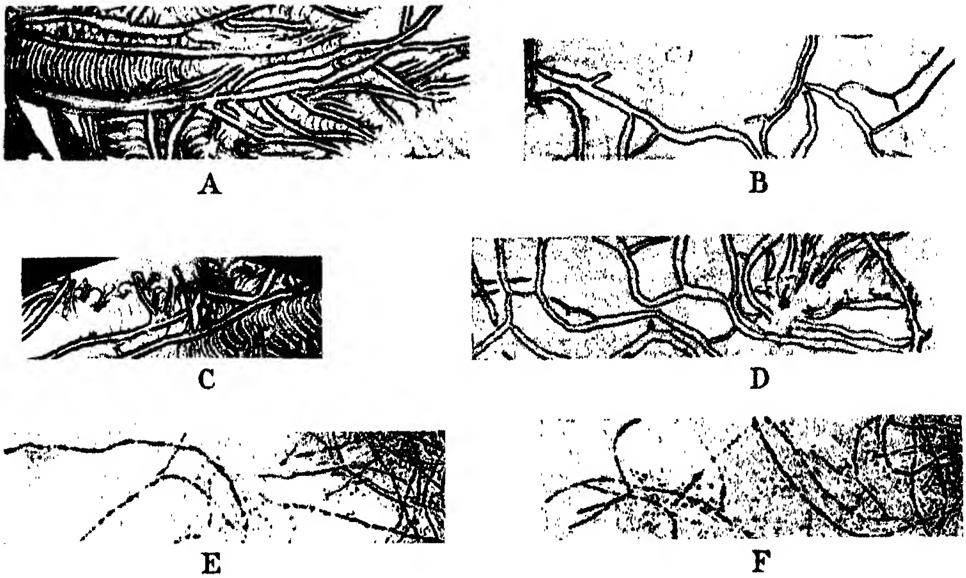

Fig. 3.

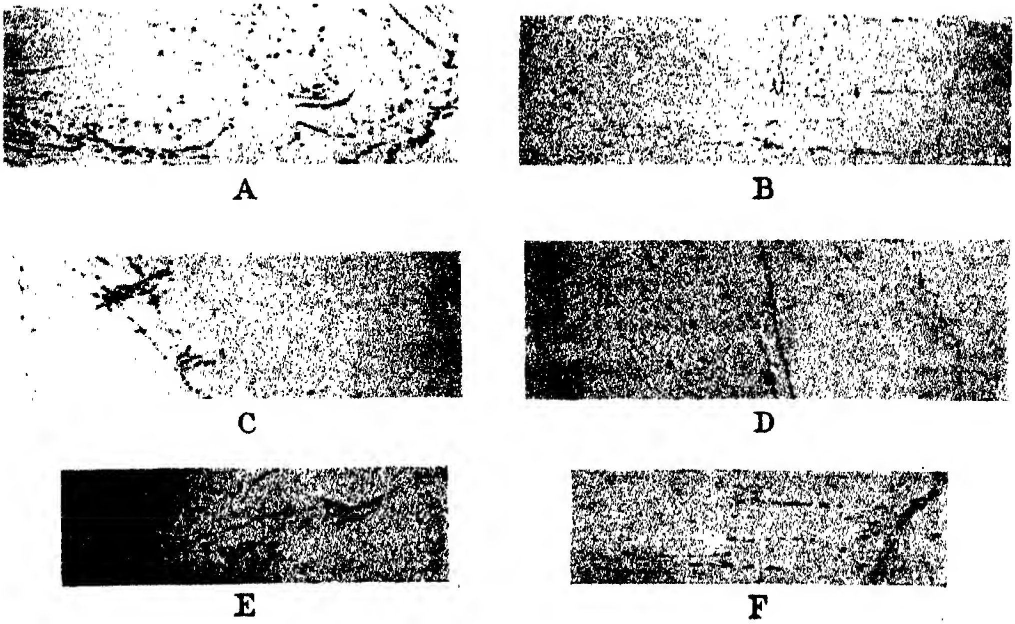

Fig. 4.

for organisms of the autoclaved parchment paper membrane. It is here to be remembered that a stout parchment paper membrane such as was being employed is less permeable than a Chamberland filter. It holds back proteins and inorganic colloids, such, for example, as the silicic acid which it is here being used to separate from the sodium chloride and excess of hydrochloric acid. It is, however, impossible to be quite certain that no chance infection occurred during the experiment, although the tubes did not show any of the usual signs of bacterial invasion.

DESCRIPTION OF PLATES.

PLATE 2.

Fig. 1.—Growths formed in Colloidal Solutions in Metastable Proportions of Colloidal Silicic Acid and Colloidal Ferric Hydrate. Magnifications about 240 diameters.

Fig. 2.—Different Views of Growths given by Metastable Admixture of Colloidal Ferric Hydrate and Colloidal Sulphur *a*. The two colloidal solutions, after heating separately to 120° C. for 10 minutes in the autoclave, were mixed in the ratio of 8 of colloidal ferric hydrate solution to 2 of colloidal sulphur *a*, when the growths shown abov appeared in a few minutes. Magnification about 240 diameters in each case.

PLATE 3.

Fig. 3.—Coarser Growths and Fibres appearing in Solutions of Colloidal Silicic Acid only. Figs. A, B, C, and D show more rapid growths produced by concentration due to evaporation between slide and cover-slip. Figs. A and C under a magnification of 140 diameters. Figs. B and D under a magnification of 500 diameters. The fibres in all four of these figures are the gel form of the diphasic system. Figs. E and F are forms appearing spontaneously in sterilised and hermetically sealed test-tubes containing colloidal silicic acid prepared aseptically from autoclaved ingredients, as described in the text. The growths were obtained after 30 days' incubation in the sealed tubes at room temperature. Magnifications for figs. E and F, 320 diameters.

Fig. 4.—Finer Forms of Growth appearing in Colloidal Silicic Acid Solution, prepared under aseptic conditions from autoclaved sodium silicate and hydrochloric acid, and kept for 30 days in hermetically sealed glass tubes. Magnification about 240 diameters. These fine nodulated fibrils are very difficult objects to photograph under the microscope, and the micro-photographs only give an idea of their appearance, and fail to reproduce the beauty of the originals.

A Contribution to our Knowledge of the Chemistry of Coat-Colour in Animals and of Dominant and Recessive Whiteness.

By H. Onslow.

(Communicated by Prof. F. G. Hopkins, F.R.S. Received January 30, 1915.)

CONTENTS.

I. Introduction.

Our knowledge of the pigments which give rise to the coat-colour of animals is very limited, and is chiefly confined to work on the melanins, an extensive bibliography of which is to be found in a monograph by Kobert* and also in a paper by von Fürth.† The dark bodies which remain as a cleavage residue after the acid hydrolysis of proteins are sometimes also called melanins, as well as the artificial products formed by the action of tyrosinase upon tyrosine.

* Kobert, 'Wiener Klinik,' vol. 27, No. 4 (1901).

† Von Fürth, 'Centralbl. für allg. Path. und pathol. Anat.,' vol. 15, p. 617 (1904).

Since the relationship of these substances to each other is still doubtful, it seems preferable to follow Gortner* in calling all the artificial black bodies humins, and to reserve the name melanin for the dark pigments occurring in the living organism.

The origin of the pigments remained in great obscurity until Landolt† suggested that melanin might be formed by a process of oxidation. But it was Bertrand's‡ discovery among plants of tyrosinase, an oxidase capable of oxidising tyrosine to a humin, that led to the present theory. Bertrand's discovery was confirmed by Biedermann§ and others who found that tyrosinase occurred among many plants and animals.

This wide distribution of tyrosinase and its obvious connection with pigment production gave rise to the current hypothesis of the mechanism of melanin formation, namely, that melanins are due to the action of an oxidase upon a colourless chromogen. This possibly takes place in two stages: (1) the formation of a cyclic compound which is split from the protein molecule by some autolytic ferment, and (2) the oxidation of the cyclic compound to a melanin by means of a specific ferment.

According to the theory of Bach and Chodat, oxidases are of a dual nature, their constituents being a peroxide (A_2O_2) and a peroxidase (P). The peroxide combines with the peroxidase to form an unstable compound (A_2PO_2). This immediately breaks down to yield

(1) An atom of "active" oxygen O.
(2) The peroxidase P.
(3) The compound A_2O.

The colourless chromogen is oxidised to a pigment by the "active" oxygen, whilst the compound A_2O is reconverted by the atmospheric oxygen to the peroxide A_2O_2, so that the cycle can be repeated indefinitely.

All attempts to ascertain the constitution of the melanin molecule have proved fruitless, mainly owing to the difficulty of isolating the pigment. The qualitative analyses do not agree in the main features and little or nothing can be deduced from the elementary analyses obtained. The mother substance of melanin has generally been supposed to be tyrosine, and Gessard‖ has shown that in one case at least tyrosine as well as tyrosinase was present in the tissues surrounding the pigment. There are, however, a number of other

* Gortner, 'Jour. Biol. Chemistry,' vol. 8, No. 4, p. 341 (1910).

† Landolt, 'Zeitschrift für Physiol. Chem.,' vol. 28, p. 192 (1899).

‡ Bertrand, 'Paris, C. R. Acad. Sci.,' vol. 122, p. 1215 (1896), and vol. 123, p. 463 (1896); 'Annales Institut Pasteur,' vol. 22, No. 5, p. 381 (1908).

§ Biedermann, 'Pflueger's Archiv für gesammte Physiol.,' vol. 72, p. 152 (1898).

‖ Gessard, 'Paris, C.R. Acad. Sci.,' vol. 136, p. 1086 (1903).

substances which might serve the purpose equally well, such as tryptophane, histidine, or the decomposition product of keratin found by Gortner,* which, though free from tyrosine, yielded Millon's reaction.

II. The Mechanism of the Action of Tyrosinase in Coloured Animals.

The work described in this paper was undertaken with a view to throwing more light upon the mechanism of the action of tyrosinase, and in it I hope to present further evidence in favour of the current hypothesis that pigmentation is the outcome of the action of an oxidase upon a colourless chromogen, and also to describe the distribution of a peroxidase in the skins of coloured rabbits. Further, I hope to show that a certain dominant white colour pattern in rabbits is due to the presence of an anti-tyrosinase or inhibitor in the skin of these animals, and that the recessive white variety is due to the lack of one or both of the oxidase and chromogen constituents of the pigment-producing system.

1. *Miss Durham's Experiments.*

At the outset of this work I was led to repeat some experiments of Miss Durham's,† carried out in 1904 for the purpose of showing the presence of a tyrosinase in the skins of young black rats, rabbits and guinea-pigs. An aqueous extract was made from the skins, and solid tyrosine as well as ferrous sulphate (as an activator) was added to the resulting reddish fluid. It was then incubated at 37° C. for some days, in the course of which dark precipitates were produced. When the skins of red guinea-pigs were used the fluid became yellow, and an orange precipitate was thrown down. In the absence of tyrosine or ferrous sulphate no coloration was produced, nor was any precipitate found. The boiled extract proved inactive.

In criticism of these experiments it seems in the first place unlikely that a black precipitate would be the first product formed. Previous investigators have found that the ferment fluid first becomes red or black on the surface where it is in contact with the air, and a precipitate only forms at the end of the reaction or on acidification. Moreover, it is not stated whether the reaction of the fluid extract was acid or alkaline. Presumably it would be acid owing to the formation of lactic acid, but it is well known that tyrosinase is most active in alkaline solutions, and that it is greatly inhibited by the presence of even a minute amount of acid. Further, it has been observed that the final colour produced by the action of tyrosinase upon tyrosine is invariably black. A reaction, therefore, which finally resulted in the pro-

* Gortner, 'Jour. Biol. Chemistry,' vol. 9, p. 355 (1911).

† Miss Durham, 'Proc. Roy. Soc.,' vol. 74, p. 310 (1904).

duction of an orange precipitate from tyrosine, as in the case of Miss Durham's red guinea-pigs, must have been of a totally different nature from any hitherto known. It is true that some tyrosine-oxidising ferments commence with a yellow or orange stage, but they finally turn black. Miss Durham also states that she extracted a tyrosinase of a less active nature from adult guinea-pigs, I, however, was entirely unable to obtain any ferment from four months' old rabbits. Riddle* also criticises Miss Durham's work, on the grounds that her extract appeared reddish, which he claims would modify the final colour. In addition to this, an oxygen carrier like oxyhæmoglobin, which is no doubt the cause of the reddish colour, would seriously interfere with the results. Finally, Gortner† says that he has made several attempts to confirm these experiments without success. Moreover he has shown that the addition of 1 mgrm. of ferrous sulphate completely inhibits the action of tyrosinase, an observation which I have been able to confirm. It seems possible, therefore, that Miss Durham's results were due to some combination of the iron with a protein molecule, or else to some degenerative change or autolysis accompanied by pigment production, such as that called by Meirowsky‡ *post-mortem* pigment production, which he showed took place even in pieces of boiled skin if they were kept for a few days in the warmth. My own results with material obtained from the skins of young black rabbits by Miss Durham's methods are in full agreement with those of Gortner. And one must conclude that the results so obtained are not due to the action of a tyrosinase as usually understood, but to some other unexplained cause.

2. *The Peroxidase Present in the Skins of Coloured Rabbits and Mice.*

It has already been pointed out that, according to the theory of Bach and Chodat, an oxidase is of a dual nature, the two constituents being a peroxide and a peroxidase, the peroxide functioning as an activator to the peroxidase, by supplying it with oxygen from the atmosphere, which may then be transferred to the chromogen or other oxidisable body. It was therefore thought possible that not only the chromogen but also the peroxide constituent of the tyrosinase had been destroyed during the process of making the extracts from the skins. In order to test this, a very small quantity of hydrogen peroxide, which has been shown to be capable of replacing an organic peroxide of an oxidase system, was added to the extract, in addition to tyrosine, and the tube was then incubated. After 12 hours its appearance

* Riddle, 'Biol. Bulletin,' vol. 16, p. 316 (May, 1909).

† Gortner, 'Trans. Chem. Soc.,' vol. 97, p. 110 (1910).

‡ Meirowsky, 'Centralbl. für allg. Path. u. patholog. Anat.,' vol. 20, p. 301 (1909); see also Königstein, 'Wiener Klin. Wochenschrift,' No. 17, p. 616 (1910).

was totally unlike that of any tube in the former experiments. A heavy charcoal-black ring, ½ inch wide, had formed in the upper portion of the fluid, while the lower portion remained unaltered. When the tube was shaken and allowed to incubate again, a fresh black ring formed on the surface of the fluid where it was in contact with the atmospheric oxygen. Occasionally in its earlier stages this black fluid had a reddish purple tinge, and a variable amount of black precipitate was always deposited after the lapse of some days. The results were the same if the tubes were kept at room temperature, but the darkening took place less rapidly. This seemed to be a true oxidation due to a peroxidase from the skin extracts in the presence of tyrosine and hydrogen peroxide. This reaction was accordingly always used as a method of testing for an inhibitor, in the manner afterwards described. Each experiment was repeated a number of times, and in every case with the necessary controls.

(a) *Material*—

The skins employed in these experiments were taken from a number of rabbits of the age of from two to four days, and during the course of the experiments over 200 young rabbits were employed. It was impossible to extract any ferment from older rabbits, owing probably to the increased toughness of their skins. Black rabbits were always used for the purpose of obtaining an active tyrosinase with which to test for the presence of an inhibitor. The nature of the tyrosinase in agouti, chocolate, orange, yellow, and blue rabbits, as well as in black mice, was also investigated. Since there is no true dominant white variety of rabbit, recourse was had to the type known to breeders as "English." This is a white animal with black eyes, patches of colour on the ears, face, and flanks, and a continuous line down the middle of the back. This pattern is said by Hurst* and Castle† to be dominant to self colour, a fact which was fully confirmed by breeding experiments carried out in connection with this research.

(b) *Methods*—

About eight black rabbits, preferably of the same litter, from two to four days old, were anæsthetised, killed, then carefully skinned by making an incision down the back, and the skins freed from subcutaneous tissue. The greatest care must be taken to remove completely any small blood-vessels that may be seen, otherwise the resulting fluid will be tinged with a trace of hæmoglobin which might vitiate the results. The skins were next thoroughly rinsed with water, dried with a cloth, weighed, and finely minced in a small mincing machine. The pulp was put into a mortar with about

* Hurst, 'Report Confer. on Genetics, Roy. Hort. Soc. London,' p. 114 (1906).

† Castle and Hadley, 'Amer. Nat.,' vol. 49, p. 23 (January, 1915).

half its weight of chloroform water and sufficient kieselguhr to make a good paste, and the whole mixture was thoroughly ground up for some time. The resulting black mass was finally transferred to a cheese cloth and the ferment fluid expressed by means of a hydraulic or efficient screw press. After being filtered through a soft paper, this fluid is white with a slight opalescence, provided the removal of the capillaries has been complete. The spectrum of oxyhæmoglobin cannot be detected, and the fluid does not show the presence of a trace of iron by the Prussian blue test. The extracts of the skins of all the other rabbits were prepared in exactly the same way, care being taken in the case of the English rabbits to cut away the black portions of the skin. A series of tubes was next prepared in the following manner: 2 c.c. of the ferment fluid were placed in each of a number of narrow test-tubes, together with three drops of an approximately 2-per-mille suspension of tyrosine in water, or other chromogen, and 0·1 c.c. of a 0·05-per-cent. solution of hydrogen peroxide.* This quantity of hydrogen peroxide cannot greatly be exceeded, since it has been shown by Bach† that larger amounts seriously inhibit the reaction. When an extract was to be tested for the presence of an inhibitor, it was added to the ferment fluid in the exact amount subsequently stated. Chloroform was employed as a preservative, since toluol, which was used by Miss Durham, if added in excess, rises to the surface and prevents the free access of oxygen. By means of very dilute sodium carbonate the contents of the tubes were carefully rendered faintly alkaline to litmus. The tubes were then plugged with cotton wool and incubated at a temperature of 37° C. As the reaction was found to have taken place in 12 hours the tubes were always examined after that time. An additional reason for doing this was because in some cases, when the oxidation had been slight, it was noticed that the colour had a tendency to fade after a longer period. Some idea of the amount of the pigment produced may be obtained from the following experiment:—

Ten cubic centimetres of the ferment fluid were incubated for 24 hours with an excess of tyrosine and 0·5 c.c. of a 0·05-per-cent. solution of hydrogen peroxide. This was acidified with 1 c.c. of 10-per-cent. sulphuric acid, and made up to 50 c.c. with distilled water. This solution was then titrated with potassium permanganate. It required 9·85 c.c. of a 0·2-per-cent.‡ solution to remove entirely the colour due to the melanin produced.

* For these experiments Merck's "perhydrol" was used.

† Bach, 'Ber. der Deutsch. Chem. Gesell.,' vol. 41, p. 216 (1908).

‡ According to Bach's method of estimating the amount of melanin, a 0·002-per-cent. solution of potassium permanganate was found sufficient. In the above experiment, however, this strength proved to be totally inadequate.

(c) *Properties—*

Experiment I: The Influence of Hydrogen Peroxide and of the Reaction of the Ferment Fluid.—The ferment fluid was added to each tube.

Chromogen added.	Appearance after 12 hours.		Remarks.
	Without H_2O_2.	With H_2O_2.	
None	—	—	Reaction faintly alkaline.
Tyrosine	—	—	Boiled.
,,	—	+ +	Reaction faintly alkaline.
,,	—	—	Reaction strongly alkaline.
,,	—	+	Reaction neutral.
,,	—	—	Reaction acid.

+ + indicates strong reaction.
+ indicates positive reaction.
— indicates no change.

From this experiment it appears that the ferment fluid lacks both a chromogen and a peroxide, for only when both these naturally occurring substances are replaced by tyrosine and hydrogen peroxide can oxidation take place; in other words, the ferment fluid contains only the peroxidase constituent of the pigment-producing mechanism. As a matter of fact, in later experiments when more practice had been obtained in extracting the ferment fluid, and when more care was taken to use the material as fresh as possible, a small amount of natural chromogen and peroxide was sometimes extracted. This was indicated by the formation of a dark grey ring in those tubes which contained either the chromogen or the peroxide, as well as in those which contained the ferment fluid alone. Tubes with a neutral reaction were only faintly grey after 12 hours, but subsequently they began to darken. Tubes with an acid reaction, however, remained permanently colourless. If alkali was present in excess, the reaction could not take place, nor did the tube containing the boiled ferment fluid show any sign of darkening. The peroxidase was found to be precipitated on saturating the fluid extract with ammonium sulphate, or on the addition of an excess of alcohol.

Experiment II: The Inhibitory Effect of Ferrous Sulphate.—The ferment fluid (prepared as previously, see pp. 40 and 41) was added to each tube, also tyrosine and hydrogen peroxide in the same quantities as in Experiment I.

Ferrous sulphate added.	Appearance after 12 hours.
None	+ +
1 mgrm.	—
0·5 „	+

+ + indicates strong reaction.
+ indicates positive reaction.
— indicates no change.

Although Miss Durham* obtained some catalytic reaction between her skin extracts and ferrous salts, yet the above experiment shows that ferrous salts inhibit the oxidation of tyrosine due to an oxidase; a result which is in agreement with Gortner's† observations upon the action of tyrosinase from meal-worms under similar conditions.

Experiment III: The Action of other Chromogens.—The ferment fluid (prepared as previously) was added to each tube.

Chromogen added.	Appearance after 12 hours.	
	Without H_2O_2.	With H_2O_2.
None	—	—
Tyrosine	—	+ +
p-cresol	+ +	+
Adrenalin*	—	+
Pyrocatechin (neutral)	+ +	+ +
Tryptophane	—	?
Pyramidone	—	—
Skatol	—	—
p-amidophenol	—	—

+ + indicates strong reaction.
+ indicates positive reaction.
— indicates no change.

* An aqueous extract of Burroughs and Wellcome's dry suprarenal glands was used.

(d) *Distribution and Nature of the Oxidase in Rabbits of Different Colours*—

An attempt was next made to discover whether the different coat-colours in rabbits were due to a corresponding difference in the oxidases which gave rise to them. For this purpose chocolate, blue and orange rabbits were experimented with. Extracts from the chocolate skins were rich in a ferment which appeared to be identical with that obtained from black rabbits. After twelve hours this ferment gave a deep black ring which gradually spread through the whole fluid, and at the end of some

* Miss Durham, 'Proc. Roy. Soc.,' vol. 74, p. 310 (1904).
† Gortner, 'Trans. Chem. Soc.,' vol. 97, p. 110 (1910).

days a black precipitate was deposited. The ferment also reacted with *p*-cresol, etc., and a dark grey colour was given when sufficient natural chromogen and peroxide had been extracted to yield the reaction without the addition of tyrosine and hydrogen peroxide. The fluid from blue rabbit skins—the dilute form of black—gave results similar in all respects, thus confirming the supposition that the pigments producing blue and black are identical. The yellow* rabbits employed carried the agouti factor, which is indicated by their white bellies. These skins yielded an extract in which no trace of coloration appeared, when either tyrosine or *p*-cresol was added as a chromogen, even after four days' incubation. Care was, of course, taken to remove the white bellies, in order to avoid the possible effects of an inhibitor similar to that occurring in the bellies of agouti rabbits (see p. 48). Many other chromogens were tried, such as tryptophane, adrenalin, pyrocatechin, pyramidone, guaiacol and other polyphenols, but no specific ferment could be detected.

Similar results were obtained when orange* rabbits were used. In these skins there is no fear of the presence of an inhibitor, as the bellies are self-coloured.

III. The Cause of Dominant Whiteness.

It is well known that white animals and flowers may be divided into two distinct classes—albinos or recessive whites and dominant whites. Dominant whites when crossed with coloured varieties invariably throw white offspring in the first generation, that is to say, they behave as a dominant to coloured varieties. Such dominant whites exist among various domestic animals, as for instance the White Leghorn fowl. The other type, albino, behaves as a simple recessive when crossed with coloured varieties, giving nothing but coloured offspring in the first generation. These albinos occur among most kinds of domestic animals, and anomalously among wild species. A further difference between the two forms lies in the fact that the eyes of dominant whites are generally more or less heavily pigmented, whereas the eyes of albinos are pink and almost entirely devoid of pigment. For the sake of clearness, albinos will be called "recessive whites" throughout the rest of this paper. Animals that are partially white or piebald may also be placed under one or other of these categories. In these animals the pattern, or, speaking more correctly, the white portions of it, are usually recessive to colour, but in certain cases, such as the "English"

* For the yellow rabbits I was indebted to Mr. J. Hammond, and for the orange rabbits, which correspond in the chocolate series to yellow rabbits in the black series, to Prof. R. C. Punnett, whose paper in the 'Journal of Genetics,' vol. 2, No. 3, p. 235 (November, 1912), gives a full account of this relationship.

rabbit, they are dominant. In man,* white and spotted negroes have for a long time been well known, but their genetic behaviour is far more complicated than that of most animals.† A simple and most interesting case, however, has lately been described, of a family of spotted negroes,‡ possessing a white skin pattern which apparently behaves as a dominant to the normal black type. It would be of the greatest interest to know whether the white skin of these individuals contains a pigment-inhibiting substance such as the one about to be described.

These two forms of whiteness and partial whiteness are visibly, and have hitherto been chemically, indistinguishable, being capable of differentiation by breeding experiments only. The chief problem therefore which presented itself was to discover whether any chemical basis underlay this difference. I propose to present evidence of the fact that dominant whiteness may be caused by the presence of an inhibitor of the pigment-producing oxidase, that recessive whiteness may be caused by the absence of either or both of the chromogen and oxidase constituents of the pigment-producing system, and finally, to make some suggestions as to the probable cause of variation in coat-colour.

1. *The "White Melanin" Theory.*

Two distinct theories have been advanced to account for dominant whiteness. The announcement by Spiegler§ that he had isolated a greyish substance from white horse-hair, which he called "white melanin," seemed to offer one possible explanation, namely, that a specific white pigment body, behaving as a dominant to the pigment of other colours, was present in the hair, so that the two forms of whiteness were due to the presence or absence of a "white melanin." This hypothesis has been criticised by Gortner.‖ He attempted to isolate this pigment by hydrolysing various keratin structures with 10-per-cent. sodium hydroxide and subsequently precipitating with hydrochloric acid. From black wool he obtained 2·45 per cent. of melanin, but from the feathers of dominant and recessive white fowls he obtained respectively 0·195 and 0·155 per cent. of a greyish brown substance. Since the yield of the grey substance from the white animals was so much smaller than the melanin from the black wool, and since in this and in other

* Pearson, Nettleship, and Usher, 'Drapers' Company Research Memoirs,' Biom. Ser. 8, 1911.

† Davenport, Publication by the Carnegie Instit., Washington, U.S.A. (1913).

‡ Simpson and Castle, 'Amer. Nat.,' vol. 47, p. 50 (January, 1913).

§ Spiegler, 'Hofmeister's Beiträge zur chem. Physiol. u. Pathol.,' vol. 4, p. 40 (1904).

‖ Gortner, 'Amer. Nat.,' vol. 44, p. 497 (August, 1910).

experiments the percentage yield from the dominant and the recessive whites was so similar, Gortner concluded that dominant whites do not possess a "white melanin" which is lacking in recessive whites; and, further, that the greyish brown substance derived from the feathers was not a pigment body at all, but merely a decomposition product of the keratin. The mere fact that the percentage of the grey substance was no larger in the dominant than in the recessive white feathers does not seem in itself to be a very strong argument in favour of the absence of the white pigment body. If this existed it might easily have been hydrolysed by the strong alkali used (10 per cent.).

But Gortner is undoubtedly correct in denying the pigment nature of the greyish brown substance. I have obtained it by Spiegler's methods from the hair of albino rabbits and white sheep, and find it to be an admixture of cholesterine and cholesterine esters from the surface of the hair and a substance resembling meta-protein derived from the hydrolysis of the keratin. The properties of this substance will be dealt with in a separate communication.

2. *The Inhibitor Theory.*

Having discarded the theory of a "white melanin," Gortner* suggested that an inhibitor might be present. He assumed that normal pigment formation is due to the oxidation of a chromogen (tyrosine) induced by the action of an enzyme (tyrosinase), and suggested that the tyrosine gives rise to (or is replaced by) a closely related substance which acts as an inhibitor or anti-tyrosinase. This substance is deposited in the epithelial cells of the skin, and the potentiality to reproduce it is transmitted to all the offspring. In support of this theory he was able to show that certain dihydroxyphenols which carry the hydroxyl groups in the meta-position to each other, such as orcin and phloroglucin, inhibit the action of certain tyrosinases upon tyrosine and other compounds. Later, Keeble and Armstrong† were of the opinion that the dominant white variety of *P. sinensis* contains an inhibitor, after the destruction of which by hydrogen cyanide the petals give strong oxidase reactions with suitable reagents. Miss Wheldale‡ also regards the pale shades of certain flowers which are dominant over the deeper shades as due to the presence in the petals of deoxidising substances such as tannin or sugar, and Atkins§ has observed and precipitated anti-oxidases in numerous

* Gortner, 'Jour. Biol. Chemistry,' vol. 10, No. 2, p. 113 (1911).

† Keeble and Armstrong, 'Journal of Genetics,' vol. 2, p. 277, No. 3 (November, 1912).

‡ Wheldale, 'Progressus Rei Botanicæ,' vol. 3, p. 457 (1910).

§ Atkins, 'Sci. Proc. Roy. Dublin Soc.,' vol. 14, Nos. 7 and 8 (1913).

plant juices. Lastly, Gortner* claims to have modified the pigmentation of the larvæ of *Spelerpes bilineatus*, by subjecting the eggs to a dilute solution of orcin, and to have increased the pigmentation by subjecting them to a dilute solution of tyrosine. Moreover, the Mendelian explanation of dominant whiteness, involving the existence of a factor which inhibits pigment-formation, even in the presence of the full pigment-producing mechanism, is sufficiently convincing to give great support to the chemical evidence available.

IV. The Tyrosinase-Inhibitor.

1. *The Presence of the Inhibitor in the Skins of English Rabbits.*

Experiment IV: The Effect of the Inhibitor in English Rabbit Skins.—2 c.c. of the ferment fluid (prepared as previously, see pp. 40 and 41), tyrosine, and hydrogen peroxide were added to each tube.

No.	Substance added.	Appearance after 12 hours.
1	None	+
2	0·5 c.c. English extract	—
3	1 c.c. English extract (boiled)	+
4	Precipitate obtained from half saturation of 1 c.c. English extract with ammonium sulphate	+
5	Filtrate from precipitate in No. 4	—
6	Precipitate obtained from full saturation of 1 c.c. English extract with ammonium sulphate	—
7	Filtrate from precipitate in No. 6	+

+ indicates positive reaction.
— indicates no change.

The English extract alone had no action on tyrosine, either before or after the addition of hydrogen peroxide.

As little as 20 per cent. of the English extract was found to be sufficient completely to inhibit the oxidation due to the tyrosinase.

It is seen that the inhibitor cannot be precipitated by half saturation with ammonium sulphate, but that it can be precipitated by full saturation.

In Nos. 4 and 6 the precipitate was dissolved in the same volume of water as that of the extract taken.

The fact that ammonium sulphate alone is not the cause of the inhibition is shown by the result of No. 7.

Attempts further to purify the inhibitor obtained from the ammonium sulphate precipitate have so far failed. The chief cause of this is the inherent

* Gortner, 'Ohio Nat.,' vol. 13, No. 3, p. 49 (1913).

instability of the inhibitor, which spontaneously decomposes in 48 hours at room temperature.

2. *Distribution of the Inhibitor in the Skins of Agouti and Yellow Rabbits.*

An attempt was made to extract a ferment fluid from agouti rabbits. On two occasions a large number of tubes were prepared from these rabbits, but in no single case was there any sign of oxidation. The most probable explanation of this seemed to be that the white belly of the wild rabbit which is well known to be dominant over self-colour, contained sufficient inhibitor to prevent oxidation. The skin of the bellies of some more agouti rabbits was therefore separated from the skin of the backs, and an extract prepared from each portion. It was now found that the skin of the backs contained an active tyrosinase apparently similar in all respects to that of black rabbits, whereas the skin from the bellies contained so much inhibitor that 20 per cent. of the extract was sufficient to prevent oxidation taking place in the ferment fluid extracted from the backs. The white bellies of yellow rabbits were found to contain a similar inhibitor.

It seems indubitable, therefore, that whenever rabbits, and probably also other animals, have dominant white coats, or coats which have a white pattern that behaves as a dominant to self-colour, the dominance is produced by the presence of an inhibitor or anti-tyrosinase in the skin of the animals in question which can prevent any colour being produced by the existing chromogenic system.

V. The Cause of Recessive Whiteness.

1. *The Distribution of Enzyme and Chromogen.*

Having shown that dominant whiteness is caused by the presence of a chemical inhibitor or anti-tyrosinase, it remained to indicate the cause of recessive whiteness. It is clear that recessive whiteness cannot be due to the same factor as dominant whiteness, for it is logically impossible that a form of whiteness caused by an inhibitor can be recessive to colour, since it is not conceivable that the union of two germ-cells, one carrying potentially a chemical inhibitor and the other the full mechanism of pigment-production, could result in a pigmented animal; unless, indeed, the potentially pigmented germ-cell also contained a factor which neutralised the effect of the inhibitor. Such a supposition is hardly an economy of hypotheses.

If, then, recessive whiteness is not caused by an inhibitor, it must be due to the lack of one or both of the factors necessary for pigment-production,

namely, the enzyme and the chromogen. Gortner* has shown that in the case of the Colorado Potato-beetle, the colour pattern of the elytron is due to a restriction of the chromogen to the coloured areas, and that the enzyme is secreted over the entire surface. This distribution of chromogen was made evident by the fact that the pigmentation did not become general when an unpigmented elytron was placed in a solution of tyrosinase, whereas a solution of tyrosine caused the elytron to become pigmented over its entire surface. But in the case of rabbits the distribution is, as will be shown, different, the tyrosinase being restricted to the pigmented areas, and entirely absent from the white areas. It was easy to demonstrate the absence of a tyrosinase, but it was not so simple to do this in the case of a chromogen, owing to the difficulty attending its extraction. An attempt was therefore made, by means of a microscopical examination of a number of white hairs, to discover whether an unoxidised chromogen was present in any of them.

2. *On the Presence of Granules in Certain White Hairs, and the Possibility of their Chromogenic Nature.*

It was observed that the medullary cells of some white hairs† contained groups of small granular bodies, which may be the same as the small, conspicuously stained bodies in colourless hair, described by Nathusius,‡ and believed by him to be structurally related to pigment granules. They could be stained with hot aqueous solutions of methyl green or methyl violet, but these stains were not permanent. Much better results were obtained by using Nissl's methylene blue diluted with four volumes of water. The hairs were placed in this solution and heated in a water-bath for 30–60 minutes, according to the coarseness of the hairs, after which they were allowed to remain in xylol for about an hour, in order to expel the air from the vacuoles and to remove the excess of stain. With fine hairs, such as mouse hairs, it was found necessary to dilute the stain twenty times to prevent the medullary cells from becoming stained too deeply. Treated in this manner the granular bodies appeared exactly like groups of bright blue pigment granules. They were about the same size ($1{\cdot}5\,\mu$) as normal granules, and were situated in groups of the same appearance and in the same position within the medullary cells. In the case of white hairs from the belly of wild rabbits, in which a little pigment is present, normal black pigment granules were found interspersed here and

* Gortner, 'Amer. Nat.,' vol. 45, p. 743 (December, 1911).

† Onslow, 'Knowledge,' New Series, vol. 11, Part V, p. 161 (May, 1914).

‡ Nathusius, 'Archiv für mikroskop. Anat.,' vol. 43, pp. 152, 153 (1894).

there among the blue granules, as if now and then one of them had become oxidised. In other hairs, such as those of white mice, the medullary cells appeared bright blue and entirely devoid of granules. Whether or not these bodies represent an unoxidised chromogen there is not sufficient evidence to say. Their presence or absence was not correlated either to dominant or recessive whiteness, but their occurrence seemed rather to be specific in nature, as may be seen from the following Table:—

White hairs containing granular bodies.	White hairs lacking granular bodies.
Albino rabbit.	Albino mouse.
Dutch rabbit.	Piebald mouse.
Angora rabbit.	*Mus sylvaticus* (belly).
English rabbit.	Piebald rat.
Himalayan rabbit.	Agouti rat (belly).
Agouti rabbit (belly).	Red squirrel (belly).
Mountain hare (winter coat).	Ermine (winter coat).
White cat.	Albino guinea-pig.
Border terrier	Piebald guinea-pig.
White foxhound.	White sheep.
Tricolor foxhound.	
Arctic fox (winter coat).	

White hairs from a white horse, a white goat, a white Pomeranian, and an albino wolf were also examined. It was very difficult to determine whether they contained any granules, for although a number of particles were plainly visible within the medulla, they did not group themselves in the form of pigment granules, and their shape and distribution were irregular.

VI. The Absence of Tyrosinase-Inhibitor and of Tyrosinase in the Skins of Recessive White Rabbits and Mice.

1. *Materials and Methods.*

Owing to some difficulty in obtaining albino and black rabbits simultaneously, preliminary experiments were made upon a variety of rabbits known as Black Dutch. These rabbits have the hindquarters pigmented and the forequarters white, patches of pigment on the head and ears, and pigmented eyes. The special convenience of such half-black, half-white animals was that the black portions of the skin, when removed from the white, could be used to procure an active tyrosinase with which to test for the presence of an inhibitor in the white portions. Recessive white rabbits with pink eyes were also tested on several occasions to make sure that absence of colour was caused, in their case as well as in that of the piebalds, by the lack of tyrosinase. Further, experiments were made with extracts

from recessive white mice, which were tested with an active tyrosinase procured from black mice of the same age, as well as with that from black rabbits. These extracts behaved in every way like those from the recessive white rabbits. The method of preparing the extracts was similar to that already described, except that the mice, on account of their relative immaturity at birth, were kept till they were more than a week old, and their skins were chopped with a knife instead of being passed through a machine.

Before ascertaining the absence of tyrosinase in recessive whites, it was necessary first to show experimentally what has already been concluded logically, namely, that no tyrosinase inhibitor can be present in recessive whites. This was clearly shown to be the case by the following experiment.

2. *Distribution of Tyrosinase.*

Experiment V: The Absence of a Tyrosinase-Inhibitor in Recessive White Rabbits.—2 c.c. of the ferment fluid (prepared as previously, see pp. 40 and 41) from black rabbits was added to each tube.

No.	Substances added.	Appearance after 12 hours.	
		Without H_2O_2.	With H_2O_2.
1	Tyrosine	—	+ +
2	Tyrosine + 2 c.c. of the extract from recessive white rabbits	—	+
3	Tyrosine + 2 c.c. normal saline solution	—	+
4	*p*-cresol + 4 c.c. extract from recessive white rabbits	+	+

+ + indicates strong reaction.
+ indicates positive reaction.
— indicates no change.

As much as 50 per cent. of the extract from recessive whites was powerless to prevent darkening, though as little as 20 per cent. of the extract from English rabbits was sufficient to inhibit the reaction completely.

No. 3 was prepared as a control by substituting normal saline for the recessive white extract, in order to indicate the effect of dilution upon the ferment fluid.

The absence of an inhibitor was confirmed by using the more delicate reagent *p*-cresol in a solution of ferment diluted with two volumes of recessive white extract, and also by testing the precipitates formed by saturation with ammonium sulphate and by the addition of an excess of

alcohol. The aqueous solutions of these precipitates were powerless to inhibit an active tyrosinase solution.

Experiment VI: The Absence of Tyrosinase in Recessive White Rabbits.—The extract from recessive white rabbits (prepared as previously, see above) was added to each tube.

No.	Substance added.	Appearance after 12 hours.	
		Without H_2O_2.	With H_2O_2.
1	None	–	–
2	Tyrosine	–	–
3	*p*-cresol	–	–
4	Adrenalin	–	–

+ indicates positive reaction.
– indicates no change.

This experiment clearly shows that the extract from recessive white rabbits not only lacks a tyrosinase but also an enzyme capable of oxidising *p*-cresol or adrenalin, although Meirowsky* reports the presence of an adrenalin-oxidising enzyme in extracts from the normal human skin. The results obtained with white mice and the white areas of the Dutch rabbits were similar in all respects to those given by the recessive white rabbits in Experiments V and VI, and clearly show that recessive whiteness is due to the absence of at least tyrosinase, and possibly of chromogen as well.

3. *The Effect upon the Skin Extracts of Dihydroxyphenols.*

The presence of what might possibly have been a second specific enzyme, with properties differing from those of tyrosinase, was observed very early in the course of the experiments, but to avoid confusion the account of it has been reserved until now. An experiment was performed to see whether phloroglucin and similar dihydroxyphenols inhibited the reaction of tyrosinase in the manner described by Gortner.†

Experiment VII: The Effect of the Extract from Black Rabbits upon Dihydroxyphenols.—Ferment fluid (prepared as previously) and tyrosine were added to each tube.

* Meirowsky, 'Centralbl. für allg. Path. u. pathol. Anat.,' vol. 20, p. 301 (1909); and Münchener Med. Wochenschrift,' vol. 58, No. 19, p. 1005 (1911).

† Gortner, 'Jour. Biol. Chemistry,' vol. 10, No. 2, p. 113 (1911).

Phenol added.	Appearance after 12 hours.	
	Without H_2O_2.	With H_2O_2.
None	—	+ +
Phloroglucin	+ +	+
Orcin	+	+
Resorcin	+	+
Phenol	—	—

+ + indicates strong reaction.
+ indicates positive reaction.
— indicates no change.

In every case in which a di-phenol was added, the ring that appeared after 12 hours was coloured more or less deeply yellow. This result suggested either that tyrosinase oxidises these di-phenols yellow, or that the di-phenols inhibit tyrosinase, and are themseves acted upon by a specific ferment or some other agent present in the solution. To prove that the oxidation was not due to tyrosinase, the skin extracts from both dominant and recessive white rabbits were tested with phloroglucin, etc., and in both cases the results were similar to those given by the coloured skins, as is shown by the following experiment.

Experiment VIII: The Effect of the Extracts from Dominant and Recessive White Rabbits upon Dihydroxyphenols.—The extracts were prepared as before, and to each tube was added the phenol to be tested.

Extract.	Phenol added.	Appearance after 12 hours.
Black rabbits	Phloroglucin	+
Recessive white rabbits	"	+
English rabbits	"	+
" "	Orcin	+
" "	Resorcin	+
Recessive white rabbits	Hydroquinone (reaction neutral)	+ +
" " "	Pyrogallol (reaction neutral)	+ +
" " "	Phenol	—

+ indicates a yellow ring.
+ + indicates a brown ring.
— indicates no change.

The fact that the recessive white extract, containing no tyrosinase, behaves in a way similar to that from black rabbits, proves that the effect on phloroglucin cannot be attributed to a tyrosinase. The reaction must be due, therefore, either to an enzyme which is specific for poly-phenols, but which has no action on tyrosine, or else to the presence of organic colloidal material

in the fluid extract, which has no effect upon mono-phenols, but which readily oxidises the more complex phenols.

Now the extract was observed to be very thermostable, and this made it doubtful whether a true enzyme were present. Bertrand* has, however, described a *laccase* capable of oxidising phenols, but with no action on tyrosine, which could be subjected to a temperature of 70° C. for 15 minutes without losing its activity. Moreover, Gortner† has described a similar enzyme which he separated from tyrosinase by bringing the mixture to boiling point, at which temperature the tyrosinase is destroyed and an active enzyme remains which oxidises quinol. "This oxidase," he adds, "is much more resistant to heat than tyrosinase, and may be heated at 100° C. for some minutes without losing much of its activity. Prolonged heating, however, gradually causes it to lose its oxidising power." This enzyme was obtained from meal-worms, but a similar one was also extracted from the tissues of various vertebrates, and notably from the skin of albino rats.

In order to test the effect of temperature on my skin extracts the following experiment was made with recessive white rabbits.

Experiment IX: The Effect of Temperature on the Oxidation of Phloroglucin by the Recessive White Extract.—The recessive white extract (prepared as previously) was added to each tube, as well as phloroglucin.

In each case the extract was heated for a period of 10 minutes at the temperature stated. Before heating, the fluid was as usual made faintly alkaline to litmus, so that little or no coagulation took place.

No.	Temperature.	Appearance after 12 hours.
1	37° C.	+
2	78	+ +
3	62	+ +
4	90	+

+ + indicates strong reaction.
+ indicates positive reaction.
— indicates no change.

The increase in the oxidation of Nos. 2 and 3 after a slight heating agrees with Gortner's statement that "long-continued boiling causes a gradual loss in the oxidising power, although a very short heating seems to increase the activity." In view of this great resistance to heat it seems extremely doubtful whether an enzyme, as generally understood, can be responsible for

* Bertrand, 'Paris, C.R. Acad. Sci.,' vol. 123, p. 463 (1896).
† Gortner, 'Trans. Chem. Soc.,' vol. 97, p. 110 (1910).

this oxidation. More probably, both in this case and in that described by Gortner, the poly-phenols are catalysed by some organic colloidal material in the fluid extract and not by a specific enzyme at all.

VII. A Possible Cause of Colour Variation.

The foregoing experiments call attention to one or two points which are worthy of further consideration, since they throw some light on the nature of the cause determining the difference between the various coat-colours of rabbits.* Chodat has shown how the colour due to the action of tyrosinase upon tyrosine and certain phenols is modified by the presence of amino-acids or polypeptides. He suggests, therefore, that the colour of a given pigment depends on the action of a particular oxidase upon different combinations of a phenol group, and an amino or colour-modifying group. Before accepting such a theory it must be clearly shown that such variations in colour are really due to a difference of the quality and not of the quantity of the pigment present. The colours concerned in the case of rabbits and mice are generally divided into black, chocolate and yellow, many of the other varieties, such as agouti and blue, being caused by different combinations and concentrations of these three colours. The so-called black pigment, however, appears chocolate by strong transmitted light, and bright yellow in very dilute solutions. Moreover, chocolate pigment appears black in concentrated solutions and yellow in dilute solutions. It is possible, therefore, that these differences of colour may be due to the concentration of the pigment in the granules and to the manner of their distribution rather than to any real qualitative difference in the pigment. Now the properties of the tyrosinase extracted from chocolate rabbits appeared identical with those of the tyrosinase from black rabbits, which seems to support this view. On the other hand, no tyrosinase could be extracted from yellow and orange rabbits, which suggests that an oxidase of some other nature is present. This failure to find tyrosinase might equally well be explained on the supposition that a very small amount of tyrosinase is necessary to produce a yellow colour in the hair, and that this was lost to such an extent during extraction that the remainder could not give a yellow colour *in vitro*. The observation of Miss Durham's† that the pigment of black, chocolate, and yellow hairs showed a marked difference in solubility when the entire hair was treated with alkali, appears at first

* Chodat, 'Archives des Sciences physiques et naturelles,' 4th period, vol. 33, p. 70 (1912).

† See paper by Bateson, 'Proc. Zool. Soc.,' vol. 2, p. 71 (1903).

sight to be opposed to this view. When, however, purified samples of the pigment were used, I was quite unable to detect any such difference.

In order to put this hypothesis to a further test, a rough experiment was devised as follows:—A few decigrams of pigment were prepared from black, chocolate, and yellow rabbits, by treating the hair according to Gortner's* method of extracting melanin, with a 0·2-per-cent. sodium hydroxide solution. The three amorphous black preparations resulting appeared identical, and their solubilities were found to be similar, that is to say, they were easily soluble in alkalies, slightly less soluble in dilute acids (N/20 HCl) and insoluble in strong acids and saturated ammonium sulphate solution; 10 mgrm. of each of the purified preparations were then dissolved in 10 c.c. of 5-per-cent. sodium hydroxide, and the solutions compared in a Dubosq colorimeter. To the eye these solutions appeared a fairly uniform yellow colour, and colorimetrically the average difference between yellow and chocolate and between chocolate and black was not more than 18 per cent. This difference can be partly accounted for by the slight variation in the colour of the solutions and the consequent difficulty of comparison, and partly by the admittedly unsatisfactory method of extracting the pigment. The similarity in the colour of the solutions is the more striking in view of the contrast in the appearance of bright yellow and of black rabbits, a difference which cannot be less than several hundred per cent. On the other hand, it is conceivable that during extraction the yellow and chocolate pigments may have been decomposed by the dilute alkali and converted into black pigment.

There is one other class of colours among rabbits which deserves attention, namely, the so-called dilute colours, such as blue, which is the dilute form of black. The pigment which gives rise to this colour is identical in appearance with that of the black rabbit, both microscopically and in solution. The tyrosinase which it is possible to extract also behaves like that of the black rabbits. To account for this difference in colour it has been suggested that the pigment granules are deposited far less abundantly in the blue hair than in the black.† A number of careful microscopical examinations have, however, convinced me that the amount of pigment deposited in the distal portion of the hairs is not appreciably less in blue and other dilute animals than it is in black.‡ The basal portions, on the other hand, contain very few granules, but the colour of an animal is given by the distal portion of the hair alone, since this is the only part of the

* Gortner, 'Jour. Biol. Chemistry,' vol. 8, No. 4, p. 342 (1910).

† Bateson, 'Mendel's Principles of Heredity,' p. 83.

‡ Onslow, 'Knowledge,' New Series, vol. 11, Part V, p. 161 (May, 1914).

hair to be seen when the coat is lying flat. The blue appearance must therefore be due to some other cause, and I believe the fact to be that in blue hairs the pigment is entirely confined to the medulla, whereas in black and other intense colours there are many hairs in which granules are distributed throughout the fibrils of the cells of the cortex. These granules within the cortex absorb the light which in dilute hairs passes through and is reflected by the air-cells or vacuoles which occupy the medulla, thus causing an increase in the white light and a consequent dilution of the colour observed. Dilute colours therefore, and perhaps other colours also, depend rather upon the distribution and intensity of the pigment than upon its chemical composition.

VIII. Summary and Conclusions.

1. Miss Durham's evidence for the existence of a tyrosinase in the skins of vertebrates is inconclusive.

2. A peroxidase can, however, be extracted from the skins of certain coloured rabbits and mice, which behaves like a tyrosinase towards tyrosine in the presence of hydrogen peroxide. It can be precipitated from solution by saturation with ammonium sulphate or by an excess of alcohol.

3. The peroxidase present in agouti, chocolate, and blue rabbits is indistinguishable in its reactions from that present in black rabbits; but no peroxidase could be extracted from yellow and orange rabbits.

4. Spiegler's "white melanin" is not a pigment substance; nor is it the cause of dominant whiteness, which is due, as has been suggested by Gortner, to the presence of an inhibitor or anti-tyrosinase in the skin.

5. *Dominant whiteness* in the English rabbit is due to the presence of a *tyrosinase-inhibitor* in the skin, which destroys the activity of tyrosinase; and the dominant white bellies of yellow and agouti rabbits are due to the same cause. The inhibitor can be precipitated by saturation with ammonium sulphate, and is destroyed by boiling or by standing for 48 hours.

6. *Recessive whiteness* in rabbits and mice is due to the *lack of the enzyme unit* of the pigment-producing system, for no tyrosinase or anti-tyrosinase could be extracted from their skins. There is not sufficient evidence to decide whether a chromogen is present or not.

7. The presence of an unoxidised chromogen might, however, serve to explain the occurrence of certain colourless granular particles which are found in the medullary cells of the hairs of some white animals. These particles are microscopically visible when stained, and in appearance very closely resemble coloured pigment granules.

8. The capacity of both white and coloured skin extracts to oxidise

dihydroxyphenols, but not mono-phenols, is more probably due to the catalysing effect of organic colloidal material than to a true enzyme as stated by Gortner. The extreme resistance to high temperatures shown by these extracts excludes the presence of an enzyme as generally understood.

9. Variations in coat-colour are due probably to a quantitative rather than to a qualitative difference in the pigment present, for the pigments isolated from black, chocolate, and yellow rabbits show very little difference either in the depth of their colour or in their chemical behaviour.

10. Blue and the other dilute coat-colours are not caused by a lack of pigment in the medulla, but by the absence of granules in the cortex, which, being present in the intense colours, absorb the light which in the dilute colours is reflected from the vacuoles.

In conclusion, the writer of this paper wishes to acknowledge his indebtedness to Mr. S. W. Cole for his invaluable suggestions and help throughout the course of the experiments, and to Prof. F. G. Hopkins for his kindness in revising the paper.

The Transmission of Infra-red Rays by the Media of the Eye and the Transmission of Radiant Energy by Crookes and other Glasses.

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(Report of Experiments carried out for the Glassworkers' Cataract Committee of the Royal Society.)

(From the Physiological Laboratory, Cambridge.)

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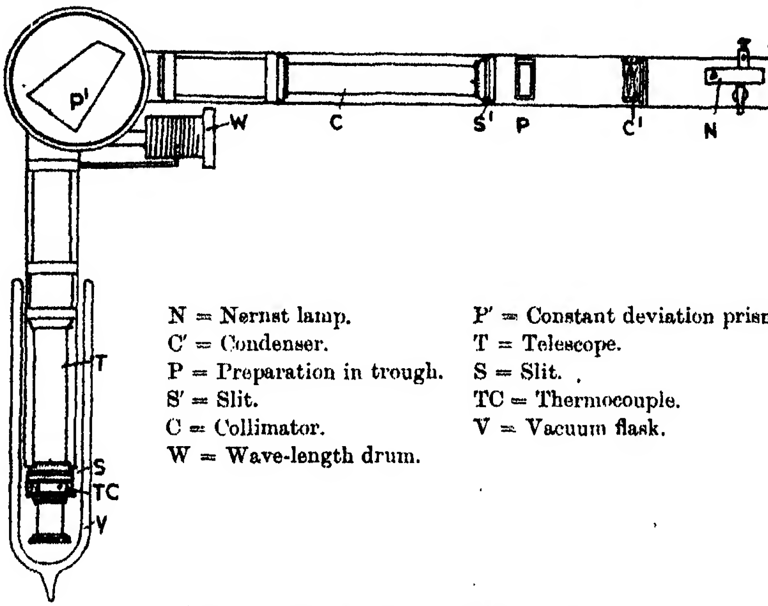
Our experiments were designed to obtain evidence on the following points:—

(1) In what amount do the infra-red radiations of different wave-length gain access to the deeper structures of the eye, the lens being particularly considered?

(2) What percentage of these radiations is absorbed in transmission through the lens?

The apparatus is shown in fig. 1; it consisted of a standard constant deviation Hilger spectrometer, which was modified in the following manner.

The eyepiece being removed from the telescope was replaced by an adjustable vertical slit, immediately behind which was mounted a delicate thermopile of 10 bismuth-silver elements.* The terminals of the thermopile were connected directly to a Paschen galvanometer† by the deflection of which the energy falling on the thermopile could be measured. The whole telescope was insulated from radiant and convected heat by a



N = Nernst lamp.
C′ = Condenser.
P = Preparation in trough.
S′ = Slit.
C = Collimator.
W = Wave-length drum.
P′ = Constant deviation prism.
T = Telescope.
S = Slit.
TC = Thermocouple.
V = Vacuum flask.

Fig. 1.—Plan of Infra-red Spectrometer.

silvered vacuum flask, the mouth of which was closed by dry cotton wool. The prism was of special dense flint, and the prism table was calibrated in wave-lengths throughout the visible and infra-red regions to λ 20,000. The collimator slit had specially curved jaws to compensate for the difference in refraction suffered by an oblique ray compared with one falling normally on to the prism surface. The condenser system was mounted on a long arm which extended beyond and in a line with the collimator. The light source was a single vertical Nernst filament, taking 100 watts, approximately. The positions and focal lengths of the lenses forming the condenser system were carefully studied, the principle employed in the construction being one that had been found by one of us to be very valuable when applied to the microscope.‡ The lens system consisted of two chief components. The first component consisted of two separate lenses which collected the rays diverging

* 'Trans. Optical Soc.,' vol. 13, p. 179.
† A Broca galvanometer was used for the earlier experiments.
‡ 'Roy. Micro. Soc.,' 1913, p. 365.

from the light source and forming a magnified inverted aplanatic image on the slit of the collimator. The function of the second component was to act as field lens; it formed a magnified inverted image of a plane situated between the two lenses of the first component on to the plane of the collimator lens. The aperture of the condenser system was purposely made considerably greater than was actually required to fill completely the aperture of the collimator. The object of this system may be indicated as follows. If a piece of plane parallel glass or a thin trough with plane sides containing fluid be interposed between a lens and the plane at which it is forming an image, but little disturbance will occur. If, however, the sides of the glass be not parallel or if there is any lens action, then considerable alteration will occur not only in the position of the image but also in its definition. As will be explained in dealing with the measurements on the lens of the eye, no matter how carefully the refracting power of the lens is neutralised, there will always be some residual refraction, particularly when waves differing from one another greatly in length are to be measured. Now one property of the condenser system employed is that it is to a considerable extent unaffected by small changes in focus brought about by weak positive or negative lenses placed between its components.

The object of our earlier experiments was to ascertain the best way of dealing with the different eye media. We found that the aqueous and vitreous humour when placed in a small trough with parallel sides gave a clear sharp image when a distant light source was looked at through the trough. With the lens and cornea this, of course, would not be the case. We tried two ways of dealing with the former. The first was to take several lenses, dry them superficially, and then squeeze them into the small trough, removing air bubbles with a small glass rod. This method was quite unsuccessful; the difference in refractive index of the different zones of the eyes was found to give a series of confused images of a distant light source. The second way was to immerse the uninjured lens in some fluid of suitable refractive index that would neutralise the convergence exerted by the lens on a parallel beam of light passing through it.

There are several groups of substances that could be used for this purpose; we had, however, to select one which, besides having the right refractive index, also showed no marked selective absorption in the infra-red region. After examining a number of oils and hydrocarbons of the paraffin series we found in carbon tetrachloride the body most suitable for our purpose. We found it to have no absorption bands over the range required, a result which confirmed Abney's* data for the same substance. Further, its refractive

* Abney and Festing, 'Roy. Soc. Proc.,' vol. 38, p 77 (1884–5).

index was nearly the same as that of the lens, the values being 1·46072 and 1·42 respectively.

It does not precipitate the proteids of the lens and yet has marked antiseptic properties. We found that if a cover was put over the trough containing the lens to prevent the evaporation of the carbon tetrachloride, the preparation remained clear and bright and could have been used on several successive days had this been necessary. We used in our experiments the eyes of the ox, for their large size was a distinct advantage for our purpose. The lens and vitreous were generally removed together by making a wide lateral incision circumferential to the globe and then carefully expressing the contents. We found this method superior to our original technique, in which we removed the lens by the ordinary operation for extraction of cataract. The lens was then carefully separated from the vitreous and was introduced into the trough by gently squeezing it between two plates of glass which were held parallel with the sides of the trough. The carbon tetrachloride was then poured in and the lens prevented from floating up to the surface by a small piece of thick copper wire which was bent so as to fit the upper edge of the lens, the ends of the wire being fastened with plasticine to the top of the trough. The remaining refractivity of the preparation was now neutralised by a concave lens of suitable power fixed outside the trough. On the other side was fastened a tinfoil diaphragm, the aperture in this being freshly cut for each preparation, only so much of the lens being used as could be simultaneously neutralised; as a rule the aperture measured between 5 and 7 mm.

Table I.—Table showing Comparative Values of Infra-red Rays of Different Length transmitted by the Lens and by an Equal Thickness of Water.

λ.	Water.	Lens.	Ratio.
7,000	11	8	0·73
7,500	18	14	0·78
8,000	26·5	21·5	0·81
8,500	35	31·5	0·90
9,000	44	41	0·93
9,500	42·5	39	0·92
10,000	51	52	1·02
10,500	71·5	72	1·00
11,000	78·5	77	0·98
11,500	48·5	46	0·95
12,000	41	45	1·10
12,500	50·5	56·5	1·12
12,750	51·5	58	1·13
13,000	44	55	1·25
13,500	14	30	2·1
14,000	2	4	2

This Table is shown plotted in fig. 2.

Preliminary observations with lens preparations made in this way showed us that the absorption bands in the infra-red corresponded very closely in position to those of water. On closer comparison, however, we found that there was in addition in the lens preparation what appeared to be a more general absorption which gradually increased in amount as one passed toward the visible spectrum. Fig. 2 is typical of the results we obtained with several different lens preparations. Several explanations occurred to us to

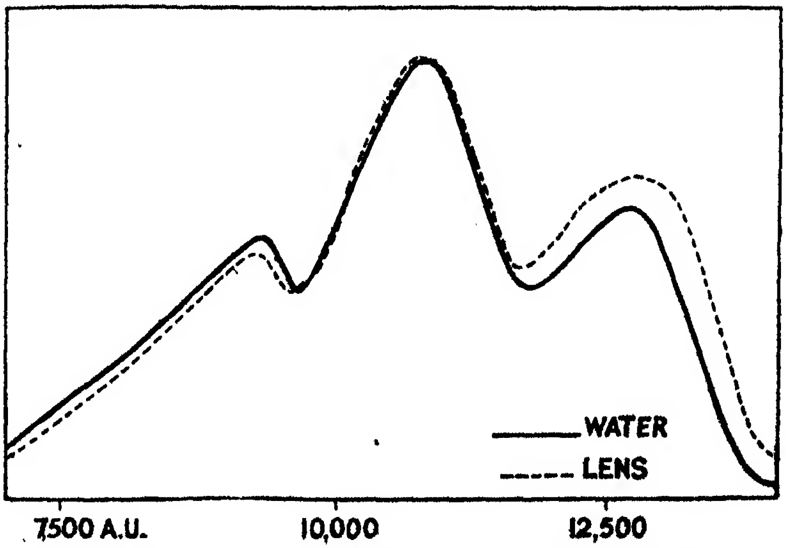


FIG. 2.—Comparison of Amount of Infra-red Energy of Different Wave-length transmitted by Lens and by an Equal Thickness of Water.

Swing of galvanometer vertical. Wave-length horizontal.

account for these results. We first supposed that there was some substance present in the lens with a very diffuse absorption band. This, however, would not fit in with the fact that the absorption band, extending as it did to 7500, would be seen in the visible spectrum and would therefore cause the lens to appear greenish in colour. Our second theory was that in spite of the special condenser system, described above, we were getting the effects of the difference in dispersion between the visible and infra-red rays, so that, while the infra-red rays in the case of the figures mentioned above were properly focussing on to the slit, some of the visible rays crossed too early and were lost. This explanation too had to be abandoned for several reasons. In no case were we able by changing the focus to get the reverse effect, *i.e.*, the visible rays giving full values and corresponding to water, and the infra-red rays giving values which fell more and more away from the water curve. A considerable change

in focus of the condenser did not alter the values at any one point. The values we obtained at different times with different lens preparations agreed with one another. At last the true cause occurred to us, namely, that the lens only contains perhaps 90 per cent. water and we were therefore comparing two unequal thicknesses of water. In making further series we therefore reduced the thickness of water from 10·15 mm. to 8·7 mm. by a thin glass plate, the same being done in examining the vitreous and aqueous humours.

Table II.—Comparative Values of Amount of Infra-red Energy of Different Wave-length transmitted by the Lens and Aqueous and Vitreous Humours and by an Equivalent Thickness of Water.

Wave-length in A.U.	Water.	Deflection of galvanometer.		
		Aqueous.	Vitreous.	Lens.
		mm.	mm.	mm.
13,500	14	13	15	22·8
13,000	44	40	50	53·5
12,500	50·5	48	56	54·1
12,000	41	38·5	44	44
11,500	48·5	45	53	49·5
11,000	78·5	75	89·5	75
10,500	71·5	68·5	83	71
10,000	51	50	59	49
9,500	42·5	38·5	46	42·5
9,000	44	40	47	38·5
8,500	35	31·5	37	31
8,000	26·5	24	30	25
7,500	18	16·5	21·5	15·5

This Table is shown plotted in figs. 3, 4 and 5.

The correspondence between the absorption curves obtained for the different eye media and for water was now nearly complete, the values given in Table II and shown plotted in figs. 3, 4 and 5 may be given as examples. It seems clear therefore that no considerable difference exists between the absorption bands of the eye media and those of water. This conclusion which we had already reached has been confirmed by finding a paper by Aschkinass,* who investigated the permeability of the eye media to red and infra-red rays.

Aschkinass first made a careful investigation of the absorption bands of water, in thicknesses from 10 μ up to 1 metre; he found bands at 0·77 μ, 1·0 μ, 1·25 μ, 1·5 μ, and 1·94 μ. The method used was that of the bolometer, in which one scale-division on the galvanometer represented 30×10^{-6} °C. Our method must be some six or seven times as sensitive as this, and the

* 'Wied. Ann.,' vol. 55, p. 401 (1895).

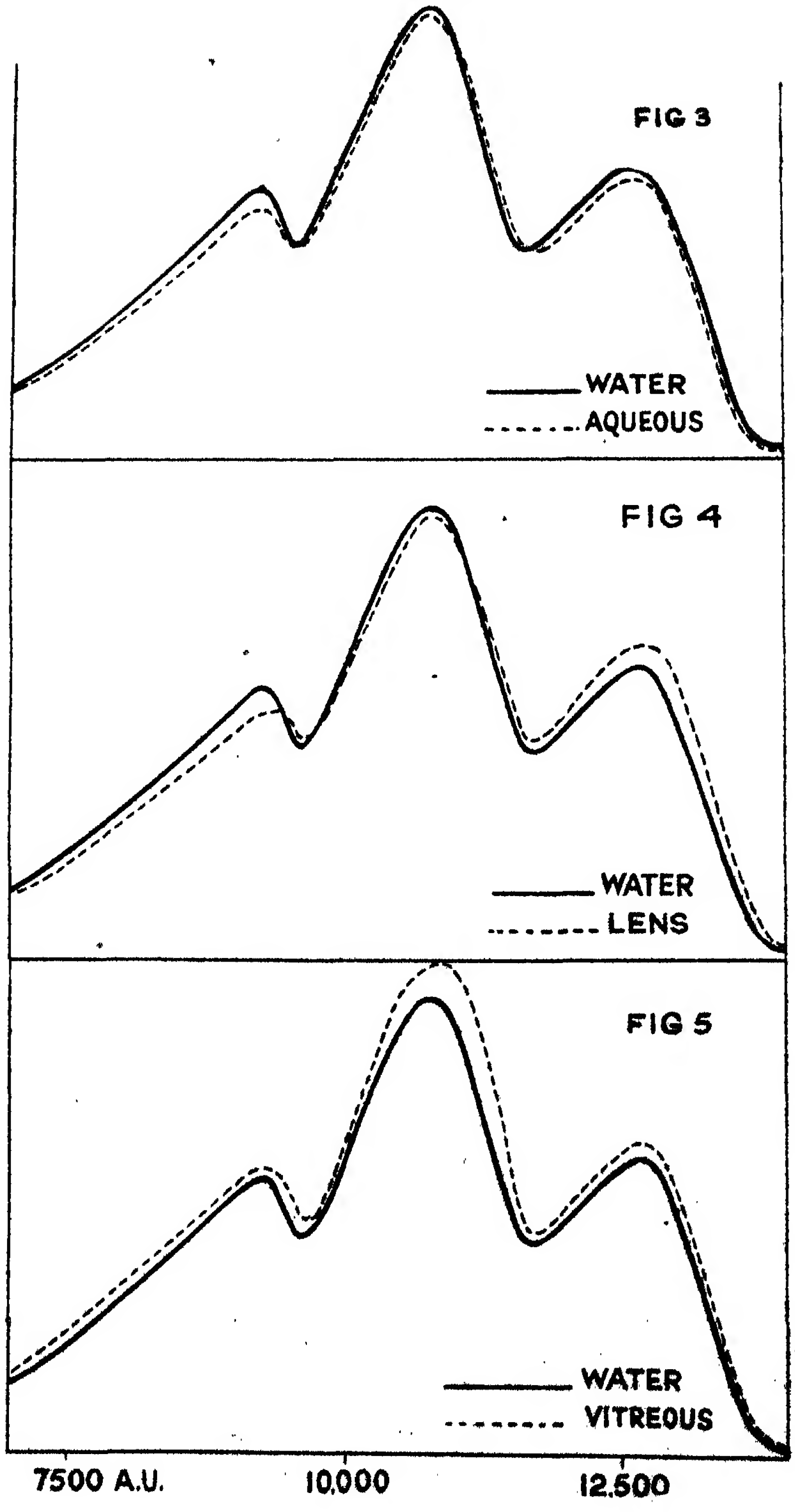


FIG 3
WATER
AQUEOUS
FIG 4
WATER
LENS
FIG 5
WATER
VITREOUS
7500 A.U.
10,000
12,500

dispersion and "over-lap" are probably better. He then investigated in order the absorption by the cornea, the aqueous, the lens, and the vitreous of the bullock's eye. In the cornea, when pressed flat, there is almost invariably some degree of cloudiness: this we also have observed, and the cloudiness probably leads to some general absorption throughout the whole range of wave-lengths. Aschkinass found that the general absorption produced by this cloudiness is greater the shorter the wave-length, and diminishes (as one might expect) considerably for the longer waves. There is no reason to suppose that in the normal eye this phenomenon causes any absorption at all (in the condition of glaucoma, however, it probably does): and Aschkinass, finding absorption bands at $1{\cdot}00\,\mu$, $1{\cdot}25\,\mu$, and $1{\cdot}50\,\mu$, comes to the conclusion that "the absorption follows the same course as for water." The same result he found, quite definitely and clearly, for the aqueous and the vitreous. With regard to the lens, the proof of the quantitative equality of the absorption to that of water was more difficult, as we have pointed out above. Aschkinass did not, as we have done, immerse the lens in some non-absorbing fluid (CCl_4) of approximately the same refractive index: he trusted simply to pressing it between two glass plates. He still found, however, that by virtue of its inhomogeneity it continued to act as a lens, and in order to deal with the absolute value of the absorption he had to apply a correction. Qualitatively, identically the same bands were seen as in the case of water, and quantitatively he came to the same conclusion as ourselves, viz., that the absorption of the lens is in no considerable way different from that of water.

The absorption of radiant heat by water has been known for some time. Julius investigated the absorption of both water and NaCl solution in small thicknesses. Abney,* using both photometric and thermometric methods, investigated very completely the absorption of water up to layers 2 feet thick. He found that water between the sodium lines and $2{\cdot}4\,\mu$ had absorption bands with the following maxima 0·580, 0·670, 0·780, 0·860, 0·970, 1·20, 1·45, 1·90, and 2·50 approximately, the first four bands being shallow except for thick layers; the last five bands being of increasing depth. Paschen† carried out a more complete investigation of the far infra-red absorption up to $10\,\mu$; he found that thin layers of water even take up a considerable portion of the incident radiation of wave-length greater than $2{\cdot}3\,\mu$. Thus a layer 0·03 mm. thick transmitted at no wave-length more than 30 per cent. of the incident energy. A layer 2 mm. thick would, therefore, be totally opaque for wave-lengths greater than $2{\cdot}3\,\mu$. This is an

* 'Roy. Soc. Proc.,' vol. 35, p. 328.

† 'Wied. Ann.,' vol. 52, p. 216 (1894).

essential fact from our point of view, for it means that the radiation reaching the lens must be of shorter wave-length than 2·3 μ, and is therefore able to pass readily through ordinary glass.

We next turned our attention to the second part of our enquiry, namely, to what extent do the various structures of the eye receive and absorb the infra-red radiations. We attacked the question in two ways, partly by direct experiment, and partly by calculation from the measured absorption of a standard thickness of water. The results obtained by the two methods agreed with one another.

Table III.—Absorption by Water in Percentage of Incident Heat Energy.

Å.U.	Thickness.	Readings.				Mean.	Log of reciprocal.
	mm.	p.c.	p.c.	p.c.	p.c.		
7,000	30·6	102·5	97	98	101	*99*	0·0044
7,500	—	95	93	96	94·5	*94·5*	0·0246
8,000	—	91·2	89·8	95	92	*92*	0·0362
8,500	—	90	92·1	91·3	90·7	*91*	0·0410
9,000	—	88	86·9	86·6	87·1	*87*	0·0605
9,500	10·5	72·3	72·6	73	71·8	*72·4*	0·1403
9,750	—	67·5	67	67·3	67·4	*67·3*	0·1720
10,000	—	74	74	73·1	74·2	*73·8*	0·1319
10,500	—	90·2	91·4	91·5	90·3	*90·9*	0·0414
11,000	—	85·5	85·3	85·4	84·5	*85·2*	0·0696
11,500	—	42·2	43	42·6	43·5	*42·8*	0·3686
12,000	—	30·7	31·3	30·3	30·4	*30·7*	0·5129
12,500	—	33·2	33·9	33·3	33·6	*33·5*	0·4750
12,750	—	33·5	33·6	34·3	33·1	*33·6*	0·4737
13,000	—	27·6	26·3	26·4	26·8	*26·8*	0·5719
13,500	3	43·1	43	43·2	43·2	*43·1*	0·3655
14,000	1	24	24·4	23·6	23·9	*24*	0·6198
14,500	—	56	5·5	5·36	5·6	*5·5*	1·2596
15,000	—	13·5	13·3	13·45	13·5	*13·4*	0·8729
15,500	—	29·4	29·2	28·2	29	*29*	0·5376
16,000	3	12·2	12·4	12·45	12·15	*12·3*	0·9101
16,500	—	16·5	16·3	16·45	16·9	*16·5*	0·7825
17,000	—	14·3	13·6	13·7	14·1	*13·9*	0·8570
17,500	—	8·35	8·6	8·4	8·3	*8·4*	1·0757
18,000	1	20·5	20·9	20·4	20·65	*20·6*	0·6861
18,500	—	52	5·1	4·8	4·95	*5*	1·3010
19,000	—	—	—	2	—	*2*	1·7000
19,500	—	—	—	2·5	—	*2·5*	1·6021
20,000	—	—	—	4·5	—	*4·5*	1·3470
20,500	—	—	—	6	—	*6*	1·2218
21,000	—	—	—	7·5	—	*7·5*	1·1249
21,500	—	—	—	7	—	*7*	1·1549
22,000	—	—	—	5	—	*5*	1·3010
22,500	—	—	—	2·5	—	*2·5*	1·6021
23,000	—	—	—	0	—	*0*	—
23,500	—	—	—	0	—	*0*	—
24,000	—	—	—	0	—	*0*	—

The absorption by water at different wave-lengths is given in Table III. The values were obtained by first measuring the deflection of the galvanometer

Table IV.—Calculated Values of Heat Radiation Penetrating the Eye in the Human Subject.

Wave-length in A.U.	I. Percentage of heat energy transmitted by cornea of that incident on cornea.	II. Percentage of heat energy reaching the anterior surface of lens of that incident on cornea.	III. Percentage of heat energy reaching the posterior surface of lens of that incident on cornea.	IV. Percentage of heat energy reaching retina of that incident on cornea.
7,000	97·5	95	95	94·8
7,500	97·5	95	94·6	91·3
8,000	97·5	94·5	93·6	89·6
8,500	97·5	94·2	93	89
9,000	97·2	93·6	91·9	86·1
9,500	94·4	85·4	76·2	48
9,750	93·6	83·1	72·5	41·2
10,000	94·5	85·8	77·2	50·3
10,500	96·6	92	89	77·6
11,000	95·9	90	85·1	67·7
11,500	89·4	71·5	58·2	15·9
12,000	86·4	63·7	42·2	7·9
12,500	87·0	65·7	44·9	9·5
12,750	87·3	65·6	44·8	10·6
13,000	85·4	61	37·7	6·55
13,500	75·0	36·4	13·4	0·24
14,000	28·5	0·72	—	—
14,500	5·5	0·00	—	—
15,000	12·9	1·1	—	—
15,500	28·0	1·37	—	—
16,000	48·2	8·7	0·73	—
16,500	53·3	12·2	1·44	—
17,000	51·4	10	0·95	—
17,500	43·5	5·6	0·30	—
18,000	20·3	0·42	—	—
18,500	4·9	—	—	—
19,000	2	—	—	—
19,500	2·5	—	—	—
20,000	4·4	—	—	—
20,500	6	—	—	—
21,000	7·6	—	—	—
21,500	7·1	—	—	—
22,000	5	—	—	—
22,500	2·5	—	—	—
23,000	0	—	—	—

This Table is shown plotted in fig. 6.

with the trough filled with water, and then measuring at the same wave-length the deflection without the water. The percentage absorption of the water only was then obtained by stating the first measurement in percentage of the second. In measuring the deflection without the water the empty trough was not used, as it would introduce an extra pair of glass–air surfaces instead of two glass–water ones. We used instead two glass plates of the same thickness as the sides of the trough clamped together with a thin film

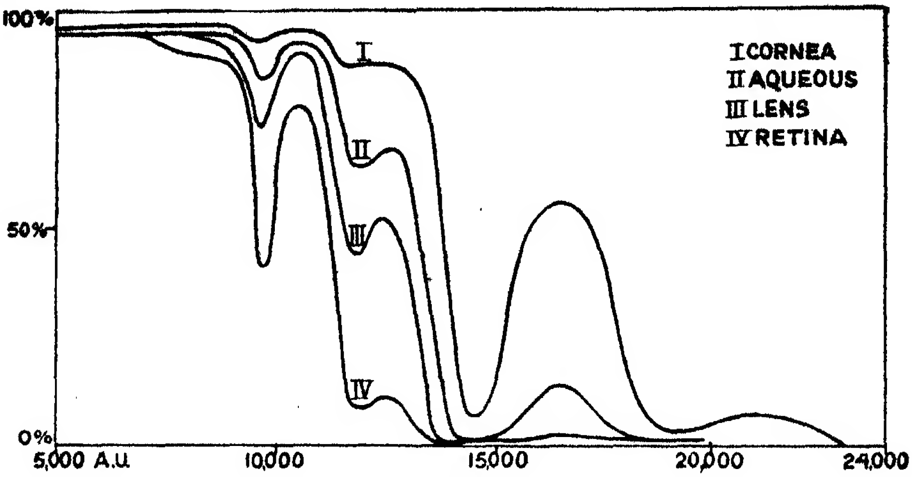


Fig. 6.

of water between. In this way the loss of light at the surfaces of the trough was allowed for. Water of several thicknesses was measured in order to give suitable values from which to calculate the absorption by thin or thick layers of eye media. From these measurements we then calculated the absorption by the different structures of the eye, using the values given in Table V for their thickness and the percentage of water contained in them.

Table V.

Structure.	Thickness.	Refractive index.	Water.	Equivalent thickness of water.
			per cent.	
Cornea	1·15	1·377	90	1·04
Aqueous humour	2·4	1·335	99	2·38
Lens centre	—	—	84	—
Lens cortex	4·05	1·39	92	3·55
Vitreous humour	15	1·34	96	14·4

(The values in the last column were calculated by multiplying the thickness of the structure by the percentage of water contained by it.)

Table VI.—Calculated Values of Heat absorbed by Cornea, Lens, and Iris.

Wave-length in A.U.	I. Percentage of heat energy absorbed by cornea of that incident on cornea.	II. Percentage of heat energy absorbed by iris of that incident in cornea.	III. Percentage of heat energy transmitted by lens of that incident on lens.	III. Percentage of heat energy absorbed by lens of that incident on cornea.
7,000	—	95	100	—
7,500	—	95	99·4	0·4
8,000	—	94·5	99·1	0·9
8,500	—	94·2	98·8	1·2
9,000	0·3	93·6	98·2	1·7
9,500	3·1	85·4	89·3	9·2
9,750	3·9	83·1	87·1	10·6
10,000	3	85·8	90·0	8·6
10,500	0·9	92	96·7	0·3
11,000	1·6	90	94·6	4·9
11,500	8·1	71·5	74·3	18·3
12,000	11·1	63·7	66·2	21·5
12,500	10·5	65·7	68·4	20·8
12,750	10·2	65·6	68·3	20·8
13,000	12·1	61	63·1	24
13,500	22·5	36·4	36·9	23
14,000	74	72	0·63	0·72
14,500	92	—	—	—
15,000	84·6	1·1	0·08	1·1
15,500	69·5	1·37	1·23	1·37
16,000	40·3	8·7	8·4	8
16,500	44·2	12·2	11·8	10·8
17,000	47·2	10	9·53	9
17,500	55·2	5·6	5·37	5·3
18,000	77·4	0·42	0·36	0·42
18,500	92·5	—	—	—
19,000	95·5	—	—	—
19,500	95	—	—	—
20,000	93		—	—

This Table is shown plotted in fig. 7.

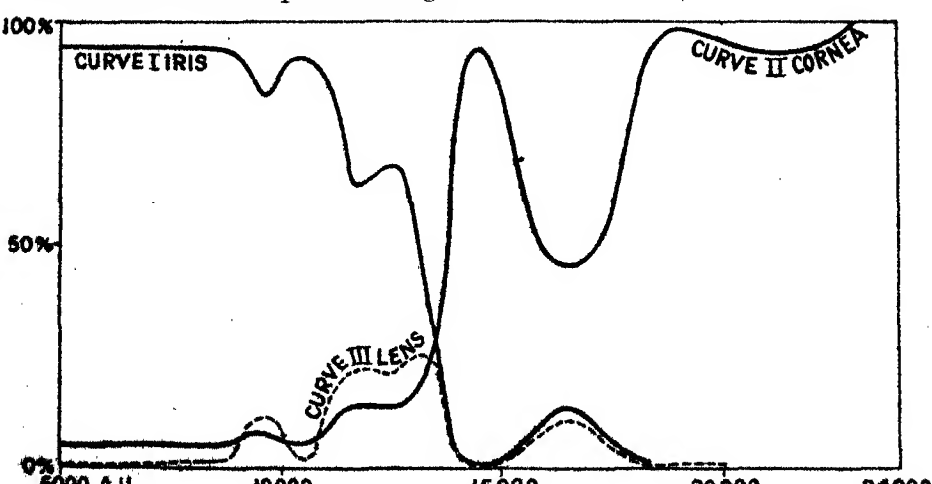


Fig. 7.

Now besides absorption by the eye structures a certain small amount is lost by reflection at the different surfaces and by scattering, since the eye media are not entirely homogeneous. Heat lost by reflection is greatest at the anterior corneal surface, being about 2·5 per cent. At the other surfaces about 0·5 per cent. is lost. The probable total loss by reflection and scattering we have assumed to be 5 per cent. The values in the above Tables are shown plotted in figs. 6 and 7. Examination of these Tables shows that the heat radiation from λ 11,000 to λ 7000 passes into the eye almost unchecked and a great deal of it reaches the retina. This entirely confirms the results obtained by Vogt mentioned above.

Now, we found the iris of the ox totally obstructed heat radiation of every wave-length which fell upon it. It therefore absorbs the same percentage radiation as that which reaches the anterior surface of the lens; that is roughly 75 per cent. of the heat radiation between λ 13,000 and the visible spectrum. The lens, on the other hand, absorbs of the radiation allowed to reach it through the aperture in the iris only a very small percentage of the incident light energy, approximately 12 per cent. Thus, in the case of the ox and the radiation from a naked Nernst filament, four times the amount of energy is absorbed per unit area by the iris as is absorbed by the lens. The difference is, of course, still greater when unit volume is considered. Now, although an actual coagulation of the lens proteins brought about in the course of time by this small amount of heat radiation is not impossible, when the conclusions of Chick and Martin* with regard to the physical chemistry of coagulation are considered, yet we think it more likely that the change is due to some interference with the nutrition of the lens caused in some way by the enormous heat-absorbing power of the iris affecting the secretion of the aqueous humour by the ciliary body, as Parsons suggests.† It would be premature to speculate what the connection between the heat stimulus on the iris and the secretion of aqueous may be, but several interesting points may, perhaps, be briefly mentioned.

Firstly, the heat radiation is probably absorbed but slightly by the pigment in the substance of the iris, by far the greater amount of energy passing through and being finally absorbed by the pigment on its posterior surface. In the case of blue-eyed individuals the pigment in the stroma of the iris is absent and the posterior pigmentary layer is alone effective in absorbing radiant energy. This means that not only does the absorbent layer come in intimate contact with the posterior chamber of the eye, but also with the

* 'Journ. Physiol.,' vol. 40, p. 404 (1910).

† 'Affections of the Eye produced by Undue Exposure to Light,' Report to Section of Ophthalmology, British Medical Congress.

processes of the ciliary body themselves. A rise of temperature of the pigment layer due to the absorption of heat must necessarily cause at the same time a rise of temperature by conduction to surrounding structures, in this the glandular elements of the ciliary body take part.

Secondly, the very intimate relationship that exists between the arterial supplies of the iris and ciliary processes may be mentioned, both coming off as branches from the circulus arteriosus major. It is possible that the lymphatic drainage is no less intimate, it is also conceivable that the vaso-motor nerves to these arteries also send glandulo-motor nerves to the ciliary processes; on these points, however, we have only the evidence of analogy with other secretory organs.

There are several remarkable features in the occurrence of glassmakers' cataract; the very long period taken for the condition to develop does not at all suggest any pathological change of an inflammatory nature, neither has any obvious change in any other structure of the eye apart from the lens been described. Thus the pupil is normal in size and reaction to light, which would not be the case if it had been the seat of any chronic inflammatory change. It would seem to us more likely therefore that the change in nutrition of the lens is one brought about by some physiological alteration in the secretory mechanism of the aqueous rather than to a pathological change. We have only to postulate a secretion of aqueous when heat falls on the iris to obtain what appears to be a plausible hypothesis of the formation of the cataract. Normally aqueous is secreted in small amounts all the time; when heat falls on the iris a larger secretion occurs, which is followed when the stimulus stops by a period of rest. This stimulus, falling regularly for long periods, in time causes the secretory mechanism to be more and more dependent on the external stimulus. The secretion becomes periodic in character and, instead of the lens receiving nourishment all the time, it only receives it at intervals, with the result that the least well nourished part of the eye suffers and cataract develops.

Whether or not the heat absorbed by the iris stimulates the secretion of aqueous humour, and how it stimulates it, whether by rise of temperature, vaso-dilatation or actual reflex stimulation, experiment alone can show. Our object in mentioning the matter here is that it seems to us to offer a feasible line of attack for future investigation.

Protection of the Eye from Harmful Radiation by Crookes Glasses.

In order to protect the eye from damage by the radiation from luminous bodies, it is necessary to remove as completely as possible the ultra-violet and infra-red rays, for these, while taking no part in the vision of external objects,

do at the same time cause injury to the eye structures that absorb them. The visual rays when present in excessive amount or when coming from a source in a position to form sharply focussed images of filaments, etc., on the retina (eclipse blindness) also do harm, and should therefore be reduced in intensity by suitable neutral grey glasses.

In the case of daylight the modifying glasses should be worn as spectacles, in the case of artificial illuminants on the other hand they should form globes, so as to limit the rays emitted to those useful for vision. For both purposes the glasses recently perfected by Sir William Crookes are ideal.

Of the many different glasses prepared by Sir William Crookes, those containing iron in the ferrous state stand prominent for their power of absorbing the infra-red rays. Three glasses of different formulæ were sent to us to be tested, and in the case of two of them we were able to contrast the specimen made by Sir William himself on a small scale with samples of a large melt by Messrs. Chance Bros. & Co., Ltd. We also obtained specimens of certain other glasses specially made for spectacles, as we thought a comparison of the properties of these with the Crookes glasses might be interesting. The glasses were examined in the following ways.

The infra-red radiation was estimated by a simple arrangement of filament, condenser, and thermopile, the latter being enclosed in a box and carefully insulated with wool from radiation from surrounding objects. A comparison of the deflection of the galvanometer with and without the glass gave the summation of the effects of all the radiation emitted by the Nernst lamp. We then limited the radiation to the infra-red region by a gelatine absorption filter which absorbed everything shorter than λ 6700. (The construction and properties of this filter will be described later.) Finally we added a trough which contained 7·5 mm. thicknesses of water.

The transparency to visual rays was measured in a simple comparison photometer, the white equivalent being obtained by comparing the absorption of the glass with that of a standardised graduated neutral gelatin wedge. Colour filters were then placed over the eyepiece which limited the spectrum to the red, green or blue as required.

The ultra-violet transmitted by the glasses was estimated by a photographic method. In front of a fast non-colour-sensitive photographic plate was placed a gelatin filter which removed the visual radiation likely to affect the plate, while it allowed the ultra-violet to pass, the dyes used in preparing the filter being methyl violet and paranitrosodimethyl aniline. Over this were placed the specimens of glass to be tested, and along the edge of the plate was placed a graduated step-wedge. Light was then allowed to fall perpendicularly on the plate holder and after exposure the plate was developed in the usual

Table VII.—Comparative Values for Percentage of Visual Infra-red and Ultra-violet Rays transmitted by Crookes Glasses and by Certain Other Glasses.

	Thickness, in mm.	Heat passed by water (10 mm. thickness).	Heat from λ700 to end of spectrum.	Red λ575–λ725.	Green λ510–λ575.	Blue λ440–λ510.	Total visual.	Ultra-violet.	Ultra-violet absorption extends to λ =
				Window Glass.					
Yellow	2·2	80	71	45	21	2	35	4	500
Blue	2·13	55	25	0·5	1	18	5	—	440
Signal green	1·55	5	22	1·5	24	40	15	4	—
				Spectacle Glass.					
Green	1·8	13	25	13	51	40	30	2·5	430
Amber	1·6	85	65	65	41	14	40	5	480
Chloroph	1·4	80	75	32	27	10	25	3	490
Furizel	1·9	65	60	26	24	7	20	2	500
Orange yellow	1·8	55	45	57	42	18	45	3	480
Euphos	1·75	95	78	53	77	40	60	5	460
				Crookes Glass.					
256	1·8	2·5	*21·5*	52	74	75	*62*	40	*354*
56	2·12	2·3	*17·5*	56	74	76	*63*	50	*340*
246	2·0	0	*2*	13	22	23	*20*	2·5	*380*
31 and 32	2·6	0	*2·5*	19	39	25	*29*	4	*362*
217	1·83	0	*4*	42	55	62	*44*	10	*347*

All values are per cent. intensity of transmitted light. Last column values are approximate only. Italic figures in last series are Crookes' values.

Table VIII.—Various Glasses. Infra-red only.

Wave-length ...	70.	80.	90.	100.	110.	120.	130.	140.	150.	160.	170.	180.	190.	200.
Yellow	52	55	54	56	56	57	58·5	59	61	61	60·5	60·5	61	60
Signal green	—	—	—	—	—	—	—	4	11	18·5	25	33	37	43
Blue	25	62	61	52	36	22	20	19·2	17	19·5	20	19	31	34
Green	—	1·5	3	8·5	17	27	38	47	56	62	66	70	75	65
Amber	76	71	73	78	80	82	84	86	86	87	90	88	88	88
Chloroph	59	68	70	76	77	82	83	85	84·5	85	81	79	78	78
Furizel	47	61	64	66	66	73	76	80	79·5	77	77	77	77·5	70
Orange yellow	59	54	53	54	61	65	67	73	76	77	77·5	76	76	67·5
Euphos	62	72	81	88	88	91	90	92	91·5	91	93	91	90	90
256	38	12	4	3	1·8	2·5	2·7	4·2	6·3	9·4	13	13	12·5	13
56	24	10	3	3	2·2	1·8	2·7	4·7	7	10·4	13·5	14·5	15	15
246	5	2	2	1·5	1	—	—	—	0·5	0·5	0·6	0·7	—	—
31	8	3·5	—	—	—	—	—	—	1	0·5	0·85	1·1	0·9	2·1
217	14	2	—	—	—	—	0·8	0·7	1·4	2·5	3·2	4	4·4	4·3

way. The depth of the silver deposit was then measured in the areas corresponding to the glasses. The densities corresponding to the step-wedge were measured in a similar manner, and the results plotted against the known values of the wedge absorption. The densities in the areas corresponding to the glasses were then referred to this curve, and the absorption stated in percentage of the incident radiation.

The infra-red radiation at different wave-lengths was obtained by the infra-red spectrophotometer used in testing the absorptions of the eye.

The results obtained by us are given in Tables VII and VIII, pp. 73–74. Comparison of the values for the different glasses shows the great infra-red absorbing power of the Crookes glasses compared with the other glasses tested. The transparency of these glasses is very considerable, while their ultra-violet absorbing power is not so great as some of the more heavily coloured orange and green glasses previously manufactured.

The formulæ of the Crookes glasses were approximately as follows:—

No. 256.*

	Per cent.
Soda flux	81
Cerium nitrate	11
Ferrous oxalate	5·4
Tartar	2
Charcoal	0·5

No. 246.†

Soda flux	90
Ferrous oxalate	10
Tartar	—
Charcoal	—

No. 217.†

Soda flux	96·8
Ferrosoferric oxide	2·85
Carbon	0·35

To facilitate comparison between the optical properties of glasses made to the above formulæ we have calculated from the values in Table VII the thickness of plate required to reduce the visual rays by 50 per cent. From

* Sir W. Crookes kindly sent us the formula of this glass for publication.

† 'Phil. Trans.,' A, vol. 214, p. 20 (1914).

this we then estimated the percentage absorption of infra-red and ultra-violet rays by such a plate.

No. of glass.	Thickness.	Visual.	Infra-red.	Ultra-violet.
	mm.			
256	2·61	50	10·7	26·4
246	0·86	50	18·6	20·4
217	1·55	50	6·61	14·3

Glass 217 would, therefore, appear to be the most efficient in removing rays likely to injure the eye. Its colour is a pale green, very pleasant to use, and the eye quickly becomes accustomed to the slight coloration. Colour matches appear to be but little affected by it.

Soil Protozoa and Soil Bacteria.

By Edward John Russell (Rothamsted Experimental Station).

(Communicated by Dr. Horace T. Brown, F.R.S. Received May 3, 1915.)

In a paper recently published by Mr. Goodey* it is definitely asserted that ciliates, amœbæ and flagellates cannot function as a factor limiting the numbers of bacteria in soils. It does not appear to me that this conclusion is justified by the experimental data given in this paper, and in view of the importance of the subject it seems desirable to bring together the main facts so far ascertained and to summarise the present position of the problem.

Soil consists of irregular mineral particles of sizes varying from about 1 mm. diameter downwards, together with a smaller proportion of organic substances of varying degrees of complexity, nutrient and other salts, and the oxides of iron, aluminium, and silicon in a form easily soluble in acids or alkalis. The action of the natural processes tends on the whole to effect intermingling of these constituents, at any rate throughout the top 6 inches.

In its physical properties soil behaves like a colloid; it possesses strong powers of absorption, and the phenomena are exactly parallel with those shown by other colloids; it influences the evaporation of water so that the curves become wholly different from those obtained from a water surface or from sand. The evidence all shows that the colloidal constituents are not segregated but are distributed over the surface of the mineral particles. Thus the soil may be looked upon as a mineral framework coated with a

* 'Roy. Soc. Proc.,' B, vol. 88, pp. 437–456.

complex mixture of easily soluble oxides of iron, aluminium, and silicon, organic substances, nutrient salts, etc., and behaving physically like a colloid.

About one-third to one-half of the volume of the soil consists of pores into which air diffuses fairly readily, so that the percentage of oxygen is almost the same as in the atmosphere, although that of carbon dioxide is higher, ranging up to 1 per cent. instead of 0·03 per cent. These pores also contain water, the volume of which normally varies from 15 to 30 per cent. of the total volume of the soil. This is mainly distributed in films over the substances coating the mineral particles, and in proportion to those coatings the volume of water is, of course, considerably greater than is here indicated.

The soil is known to be inhabited by numbers of bacteria, eelworms, Vermes and numerous other organisms of higher orders and visible dimensions; all these lead active lives. Recently it has been shown in the Rothamsted Laboratory that a protozoan fauna also exists, some members at least of which are leading a trophic existence. The investigations on the partial sterilisation of soil indicate that the activity of these trophic forms is one of the factors limiting the number of bacteria in the soil and consequently the amount of decomposition they effect. It is this conclusion that is controverted by Mr. Goodey in his recent paper.

The experimental results leading up to this conclusion are as follows* :—

1. Partial sterilisation of soil, *i.e.* heating to a temperature of 60° C. or more, or treatment for a short time with vapours of antiseptics such as toluene, causes first a fall then a rise in bacterial numbers. The rise sets in soon after the antiseptic has been removed and the soil conditions are once more favourable for bacterial development; it goes on till the numbers considerably exceed those present in the original soil.

2. Simultaneously there is a marked increase in the rate of accumulation of ammonia. This sets in as soon as the bacterial numbers begin to rise, and the connection between the two quantities is normally so close as to indicate a causal relationship; the increased ammonia production is, therefore, attributed to the increased numbers of bacteria. There is no disappearance of nitrate; the ammonia is formed from organic nitrogen compounds.

3. The increase in bacterial numbers is the result of improvement in the soil as a medium for bacterial growth and not an improvement in the bacterial flora. Indeed the new flora *per se* is less able to attain high numbers than the old. This is shown by the fact that the old flora when reintroduced into partially sterilised soil attains higher numbers and effects more decomposition than the new flora. Partially sterilised soil plus 0·5 per cent. of untreated

* The details are given in two papers by Russell and Hutchinson in 'Journ. Agric. Sci.,' vol. 3, pp. 111–144 (1909), and vol. 5, pp. 152–221 (1913).

soil soon contains higher bacterial numbers per gramme and accumulates ammonia at a faster rate than partially sterilised soil alone.

4. The improvement in the soil brought about by partial sterilisation is permanent, the high bacterial numbers being kept up even for 200 days or more. The improvement, therefore, did not consist in the removal of the products of bacterial activity, because there is much more activity in partially sterilised soil than in untreated soil. Further evidence is afforded by the fact that a second treatment of the soil some months after the first produces little or no effect.

It is evident from (3) and (4) that the factor limiting bacterial numbers in ordinary soils is not bacterial, nor is it any product of bacterial activity, nor does it arise spontaneously in soils.

5. But if some of the untreated soil is introduced into partially sterilised soil, the bacterial numbers, after the initial rise (see (3)), begin to fall. The effect is rather variable, but is usually most marked in moist soils that have been well supplied with organic manures; *e.g.*, in dunged soils, greenhouse soils, sewage farm soils, etc. Thus the limiting factor can be reintroduced from untreated soils.

6. Evidence of the action of the limiting factor in untreated soils is obtained by studying the effect of temperature on bacterial numbers. Untreated soils were maintained at 10°, 20°, 30° C., etc., in a well moistened aërated condition, and periodical counts were made of the numbers of bacteria per gramme. Rise in temperature rarely caused any increase in bacterial numbers; sometimes it had no action, often it caused a fall. But after the soil was partially sterilised the bacterial numbers showed the normal increase with increasing temperatures. Similar results were obtained by varying the amount of moisture but keeping the temperature constant (20° C.). The bacterial numbers in untreated soil behave erratically and tended rather to fall than to rise when the conditions were made more favourable to trophic life; on the other hand, in partially sterilised soil, the bacterial numbers steadily increased with increasing moisture content. Again, when untreated soils are stored in the laboratory or glasshouse under varying conditions of temperature and of moisture content the bacterial numbers fluctuate erratically; when partially sterilised soils are thus stored the fluctuations are regular.

7. When the curves obtained in (6) are examined it becomes evident that the limiting factor in the untreated soils is not the lack of anything* but the presence of something active.

* The soils included fertile loams well supplied with organic matter, calcium carbonate, phosphates, etc.

8. This factor, as already shown, is put out of action by antiseptics and by heating the soil to 60° C., and once out of action it does not reappear. Less drastic methods of treating the soil put it out for a time, but not permanently: *e.g.*, heating to 50°, rapid drying at 35°, treatment with organic vapours less toxic than toluene (*e.g.*, hexane), incomplete treatment with toluene. In all these cases the rise induced in the bacterial numbers per gramme is less in amount than after toluene treatment and is not permanent; the factor sets up again. As a general rule, if the nitrifying organisms are killed the limiting factor is also extinguished; if they are only temporarily suppressed the factor also is only put out for a time.

9. The properties of the limiting factor are:—

(*a*) It is active and not a lack of something (see (7)).

(*b*) It is not bacterial (see (3) and (4));

(*c*) It is extinguished by heat or poisons, and does not reappear if the treatment has sufficed to kill sensitive and non-spore-forming organisms; it may appear, however, if the treatment has not been sufficient to do this.

(*d*) It can be reintroduced into soils from which it has been permanently extinguished by the addition of a little untreated soil.

(*e*) It develops more slowly than bacteria, and for some time may show little or no effect, then it causes a marked reduction in the numbers of bacteria, and its final effect is out of all proportion to the amount introduced.

(*f*) It is favoured by conditions favourable to trophic life in soil, and finally becomes so active that the bacteria become unduly depressed. This is one of the conditions obtaining in glasshouse "sick" soils.*

It is difficult to see what agent other than a living organism can fulfil these conditions. Search was, therefore, made for larger organisms capable of destroying bacteria, and considerable numbers of protozoa were found. The ciliates and amœbæ are killed by partial sterilisation. Whenever they are killed the detrimental factor is found to be put out of action, the bacterial numbers rise and maintain a high level. Whenever the detrimental factor is not put out of action the protozoa are not killed. To these rules we have found no exception. Further, intermediate effects are obtained when a series of organic liquids of varying degrees of toxicity is used in quantities gradually increasing from small ineffective up to completely effective doses. The detrimental factor is not completely suppressed but sets up again after a time, so that the rise in bacterial numbers is not sustained. But the parallelism with ciliates and amœbæ is still preserved: they are completely killed when the detrimental factor is completely put

* This is dealt with fully in 'Journ. Agric. Sci.,' vol. 5, pp. 27–47, 86–111 (1912).

out of action; they are not completely killed, but only suppressed to a greater or less degree, when the detrimental factor is only partly put out of action.*

Now this parallelism between the properties of the detrimental factor and the protozoa is not proof that the protozoa constitute the limiting factor, but it affords sufficient presumptive evidence to justify further examination. The obvious test of adding cultures of protozoa to partially sterilised soil was made, but no depression in bacterial numbers was obtained, instead there was sometimes a rise. But in view of the history of investigations on malaria and other protozoan diseases no great significance was attached to this early failure.

No attempt had been made in any of the above experiments to identify the protozoa or even to ascertain whether any particular form existed in the soil in the trophic state or as cysts. The variety of forms was considerable, and it soon became evident that a definite protozoological survey of the soil was required.

This was accordingly put in hand. In order to give the survey as permanent a value as possible the investigations were not confined to the narrow issue whether soil protozoa do or do not interfere with soil bacteria, but they are put on the broader and safer lines of ascertaining whether a trophic protozoan fauna normally occurs in soil, and, if so, how the protozoa live, and what is their relation to other soil inhabitants.

The first experiments, made by Goodey,† indicated that the protozoa were present only as cysts. Subsequent investigations, however, by Martin and Lewin have established the following conclusions.‡

1. A protozoan fauna in a trophic state normally occurs in soils.

2. The trophic fauna found in the soil differs from that developing when soil is inoculated into hay infusions: the forms which appear to predominate in the soil do not predominate in the hay infusions, and *vice versa* the forms predominating in the hay infusions do not necessarily figure largely in the soil.

3. The trophic fauna is most readily demonstrated, and is therefore presumably most numerous, in moist soils well supplied with organic manures, *e.g.*, in dunged soils, greenhouse soils, sewage soils and especially glasshouse "sick" soils.

It is obvious that the protozoa which live largely on bacteria must function as a factor limiting the numbers of bacteria. The problem is therefore reduced

* Buddin, 'Journ. Agric. Sci.,' vol. 6, pp. 417–451 (1914).

† Goodey, 'Roy. Soc. Proc.,' B, vol. 84, p. 165 (1911).

‡ Martin and Lewin, 'Phil. Trans.,' vol. 205, pp. 77–94 (1914), and 'Journ. Agric. Sci.,' vol. 7, pp. 106–119 (1915).

to finding out how numerous they are and how their activity varies with the varying conditions obtaining in the soil.

Considerable difficulties arise in attempting to enumerate the protozoan fauna, and the line of attack adopted in our laboratory is to study the natural history of the trophic forms in the soil. But the attempt at enumeration has been made recently by Cuningham* in Löhnis's laboratory, with the following results:

4. Tentative minimum estimates made by a dilution method show that the trophic forms are to be numbered at least in thousands per gramme of soil.

(Bacteria commonly occur at the rate of 4 to 10 millions per gramme of soil).

5. A protozoan fauna introduced under suitable precautions into partially sterilised soil effected a considerable reduction in bacterial numbers.

This is as far as the protozoological investigations have gone at present. Cuningham's experiments are being repeated in our laboratory. For the rest, the work is not in a sufficiently advanced state to justify any conclusions as to the part played by the protozoa in the soil, but it has definitely revealed the presence of a trophic fauna and shows that the forms are of considerable interest.†

We can now turn to the criticism urged by Goodey which in his view is sufficiently cogent to upset these conclusions.

Goodey inoculated cultures of various Colpoda (*C. cucullus*, *C. maupasii*, *C. steinii*), a Vorticella (*V. microstoma*), and an unidentified amœba and a flagellate, into partially sterilised soils free from protozoa, and made periodical counts of the numbers of bacteria. The numbers fell off, but not to any greater extent than in similar soils to which no additions of protozoa were made. He therefore concludes that ciliates, amœbæ, and flagellates cannot be included in the biological factor limiting the number of bacteria in soil.

Two objections can be urged against Goodey's experiments.

1. The organisms inoculated into the soil are in the main those which figure largely in cultures made by adding soil to hay infusions. It has already been shown, however (*v. supra*), that the *culture* fauna is distinct from the *trophic soil* fauna. There is therefore no evidence that the normal soil fauna was put back into the partially sterilised soil: on the contrary it apparently was not. Nor is there evidence, except perhaps in one case, that the added organisms survived at all.

2. The difficulty of securing an adequate control is very great and does not appear to have been overcome. When a soil is partially sterilised either by

* 'Centr. Bakt. Par.,' August 1914, and 'Journ. Agric. Sci.,' vol. 7, pp. 49–74 (1915).

† See for example, the paper by Thornton and Smith, "On Certain Soil Flagellates," 'Roy. Soc. Proc.,' B, vol. 88, pp. 151–165 (1914).

heat, antiseptics, or prolonged storage, other changes are produced besides the destruction of the limiting factor and the protozoa. Some ammonia is formed and the amount of soluble matter is increased—both evidence of a change in the soil constituents—and within a few days after remoistening great numbers of bacteria and of their decomposition products accumulate.

Thus the two systems are:—

(1) Untreated soil containing normal numbers of bacteria and protozoa.

(2) Partially sterilised soil, changed somewhat, and containing abnormal numbers of bacteria and accumulation of their products.

It is obvious that the addition of protozoa to (2) does not make it equal to (1), even if the added protozoan fauna were identical with that in (1) and had an equal chance of growth. But Cuningham shows it has not, for he finds that the development of protozoa in a medium containing exceptionally large numbers of bacteria is considerably hindered. It is significant also that some 5 per cent. of untreated soil has to be added to partially sterilised soil in order to reintroduce the factor detrimental to bacteria. Nor is the apparently simple case of introducing a protozoan fauna into partially sterilised soil much better. The protozoa are not obtained in pure culture alone; they are added along with hay infusion and bacteria. Thus the two systems are:—

(1) Partially sterilised soil containing high numbers of bacteria.

(2) Partially sterilised soil containing high numbers of bacteria + hay infusion + added bacteria + protozoa.

These considerations show that no clear issue is obtained between soil protozoa on the one hand and soil bacteria on the other. Goodey's failure to observe any reduction in numbers in the circumstances cannot, therefore, be taken to justify the conclusion that ciliates, amœbæ, and flagellates do not limit the number of bacteria. Until more is known of the kinds of protozoa occurring in the trophic state in the soil, and of their life-history in the soil, it will not be possible to lay much stress on the negative results of re-infections: on the other hand, Cuningham's experiments indicate that positive results may be looked for in the near future.

The Chromosome Cycle in Coccidia and Gregarines.

By Clifford Dobell and A. Pringle Jameson.

(Communicated by J. Bretland Farmer, F.R.S. Received May 13, 1915.)

Despite the large amount of work which has already been devoted to the study of the Coccidia and Gregarines, very little indeed is known definitely about the behaviour of the chromosomes in these Protozoa. Not only has the chromosome cycle been left uninvestigated and undescribed in the majority of these organisms which have hitherto been studied, but the very existence of chromosomes in the nuclear divisions at many stages in the life-history of certain forms has even been denied; and the most contradictory and unsatisfactory accounts have been given of that most important phase in the life-cycle of the chromosomes—the phase of meiosis, or reduction.

In order to fill up this gap in our knowledge of the Sporozoa, we have made —during the last few years—a very detailed study of the chromosomes of a coccidian and a gregarine. One of us (C. D.) has investigated the coccidian* *Aggregata eberthi* Labbé, whilst the other (A. P. J.) has studied the gregarine *Diplocystis schneideri* Kunstler. Careful investigation of these two organisms has shown that the nuclear divisions at all stages in the life-histories are mitotic, and that the chromosome numbers are remarkably constant.

As our results are, we believe, quite definite and conclusive, and as the publication of them in full is likely to be unavoidably delayed for some time, we think it desirable to place them on record. Complete accounts of the life-histories of *Aggregata* and *Diplocystis* we hope to publish separately elsewhere. We shall here deal only with the essential facts which we have established concerning the chromosomes of these two forms.

1. *The Chromosomes of* Aggregata eberthi.

The life-history of this coccidian comprises a sexual generation which takes place in the body of a cuttle-fish (*Sepia officinalis*), and an asexual generation in the body of a crab (*Portunus depurator*). These generations are of the usual coccidian type. In the sexual cycle, male and female individuals ("microgametocytes" and "macrogametocytes") are formed, which give rise to microgametes and macrogametes respectively. Each of the latter is fertilized by one of the former; and after the union of the two nuclei, the zygote nucleus divides many times to form the nuclei of the numerous

* That this organism is really a coccidian and not a gregarine has already been shown (*cf.* Dobell, 1914).

sporoblasts. The uninucleate sporoblasts are then converted into spores, within each of which three uninucleate sporozoites are finally formed by further divisions of the nucleus and differentiation of the cytoplasm. In the asexual cycle, the sporozoites, after escaping from the spores, grow into large schizonts. By repeated divisions of the nucleus, each schizont becomes multinucleate, and finally breaks up into a very large number of uninucleate merozoites. When the latter leave the crab and enter the body of the cuttle-fish, they grow into the male and female individuals of the sexual cycle—thus completing the life-history.*

We may begin the history of the chromosomes with a description of these bodies in the male parasite. During the period of growth, the nucleus passes through a series of very complex stages which need not be described here. At the end of this period the first nuclear division takes place. This division begins as an ordinary mitosis, but ends as a multiple mitosis of a peculiar type. In the prophases, six filamentar chromosomes are formed from the spireme. At diakinesis they can be clearly seen and counted. They consist (fig. 1, A) of one very long chromosome (*a*) and one very short chromosome (*f*), the remaining four (*b–e*) forming a regular series of intermediate sizes. During the period of the first division, the karyosome disintegrates and disappears. It plays no part in the formation of the chromosomes.

As the chromosomes pass on to the equatorial plate of the first spindle, they shorten and thicken until they become almost spherical. They preserve, however, at this and all subsequent stages, their characteristic size-relations to one another (fig. 1, B, *a–f*). At the metaphase they divide by constriction, a daughter-group of six differentiated chromosomes passing to each pole of the spindle (fig. 1, C). In the late anaphases the chromosomes *again become filamentar.* The asters at the poles of the spindle divide many times in succession, and each time all the chromosomes split longitudinally. A complicated polyaster figure is thus formed, from which the chromosomes finally emerge in groups of six (fig. 1, D, *a–f*). Each group enters into the formation of a resting nucleus at the periphery of the organism. After this first multiple division, the nuclei divide many times in succession by ordinary bipolar mitosis—the typical set of six chromosomes being recognisable at each division. The smallest nuclei finally formed enter into the microgametes. No halving of the chromosomes takes place, therefore, in the formation of these. Each microgamete nucleus receives a typical set of six chromosomes, like those formed for the first division.

The macrogamete is formed, in the typical coccidian manner, by each female individual being transformed into a single gamete. The nucleus of

* See Siedlecki (1898), Léger and Duboscq (1908), Dobell (1914).

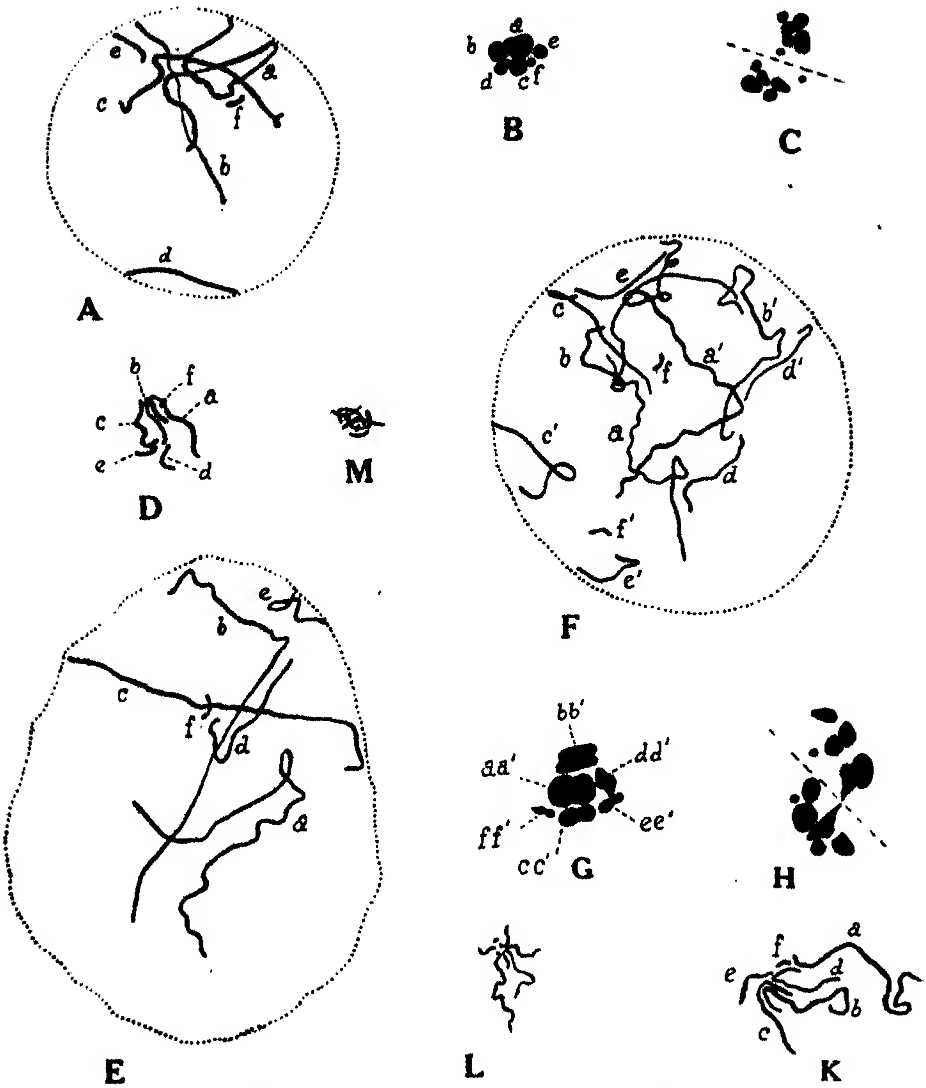


FIG. 1.—Chromosomes of *Aggregata eberthi*.

The figures are tracings (of chromosomes only) from detailed drawings made with the camera lucida at a magnification of 2500 diameters. The letters *a–f* (or *a′–f′*) indicate throughout the six chromosomes forming the haploid group, or typical chromosome complex, of *Aggregata*. The chromosomes are lettered consecutively in order of magnitude—from *a* (the largest) to *f* (the smallest).

A. Nucleus of male—prophase of first division. B. Equatorial plate—first division of male. C. Early anaphase groups at first division in male. D. Chromosome group entering into resting nucleus—end of first (multipolar) division of male. E. Chromosomes in macrogamete nucleus before fertilization. F. Chromosomes in zygote nucleus —prophase of first division. G. Double (paired) chromosomes on equatorial plate— first spindle of zygote nucleus. H. Early anaphase groups—first spindle of zygote. K. Chromosome group entering into resting nucleus—end of first (multipolar) division of zygote. L. Chromosome group—anaphase of later division (bipolar) of zygote. M. Chromosome group on equatorial plate of second division in spore.

the female passes through a complex series of stages similar to those occurring in the male; and these, likewise, culminate in the formation of chromosomes, though no nuclear division takes place before fertilization. Here, again, the chromosomes can be clearly counted, and consist of six filaments differing in size from one another (fig. 1, E, *a–f*) exactly as in the male. The karyosome breaks up and disintegrates; and, as in the male, plays no part in forming the chromosomes.

Before fertilization the chromosomes break up within the nucleus of the macrogamete. The microgamete then enters, and a series of very complex nuclear changes ensues. These include the formation of a so-called "fertilization spindle" characteristic of the Coccidia. At the end of these changes the zygote nucleus divides—the division beginning like an ordinary mitosis and then becoming multiple, as in the first division in the male. The behaviour of the chromosomes during this division is very remarkable. From the spireme 12 chromosomes are formed, which can be clearly counted at diakinesis (fig. 1, F). They consist of two homologous sets of six chromosomes each—each set (*a–f*, *a′–f′*) consisting of six filaments differentiated as regards length from one another. The two sets clearly represent the two groups of chromosomes which the zygote nucleus has received from the male and female pronuclei.

When the achromatic spindle has been formed for the first division of the zygote nucleus, the 12 chromosomes pass on to its equator. As they do so they shorten and thicken until they become almost globular. At the same time they associate in homologous pairs—*a* with *a′*, *f* with *f′*, etc.—so that six bivalent or double chromosomes finally come to lie on the equatorial plate (fig. 1, G). At the metaphase, disjunction of the temporarily united chromosomes takes place, without any splitting of the individual chromosomes; so that a group of six differentiated chromosomes passes towards each pole of the spindle (fig. 1, H). The rest of this division resembles the first division in the male. The asters divide, and the chromosomes become filamentar and split lengthwise. From the polyaster figure so formed the chromosomes emerge finally in sets of six (fig. 1, K) and enter into the formation of as many resting nuclei as there are sets of chromosomes.

The subsequent nuclear divisions of the zygote all take place by ordinary bipolar mitosis. At each division a typical set of six chromosomes (fig. 1, L) is present—one such set entering, therefore, into the nucleus of each sporoblast. A similar set of six chromosomes can be counted at each of the two mitotic divisions which take place within the spore to form the sporozoite nuclei (fig. 1, M).

During the development of the sporozoite into a schizont, the nucleus

passes through a long series of stages closely similar to those which occur in the young sexual forms. We have not been able to study the nuclear divisions of the schizont in such great detail as we have those of the sexual parasites: but it is evident from our results, taken together with those of Léger and Duboscq (1908), who have previously described these stages, that the number of chromosomes is six throughout the entire asexual cycle. The first division of the schizont nucleus is by multiple mitosis, the later divisions by ordinary mitosis into two—as in the sexual forms. The typical size-relations of the six chromosomes are probably recognisable throughout. (We have not been able to study every division, but those which we have all appear quite typical.)

From the foregoing description, it will be clear that in *A. eberthi* the chromosomes are six in number at every nuclear division in the life-history with one exception. This is the division of the zygote nucleus immediately succeeding fertilization. There are here 12 chromosomes, which become halved to six in the course of this division. Reduction thus occurs immediately after fertilization—not during gametogenesis. The six chromosomes must be regarded as representing the haploid number—the diploid number (12) being present in the zygote nucleus only, while its division is a reduction division. Since the haploid number (6) occurs in both the sexual and the asexual cycle, there is thus no differentiation in respect of chromosome number in the two generations.

2. *The Chromosomes of* Diplocystis schneideri.

The life-history of *D. schneideri* is comparatively simple. It is passed in a single host—a cockroach. We have studied it chiefly in *Periplaneta americana*, but have found it also in *Stylopyga orientalis.* At a very early stage in their development the parasites unite in pairs in the gut wall of their host, and then fall into the body cavity. After a considerable growth period in the associated condition, they form gametes. The nucleus of each member of the pair gives rise, by repeated mitoses, to a great number of very small nuclei—each of which finally becomes the nucleus of a gamete. The partition separating the two individuals (gamonts) has by this time disappeared; and the gametes, when fully formed, fuse in pairs. The nucleus of the zygote (sporoblast) gives rise to eight daughter nuclei which become the nuclei of the eight sporozoites formed within each spore. The sporozoites develop within another cockroach into body-cavity parasites once more, and the life-cycle is thus completed.

Although the mitotic figures at certain stages are very small, the behaviour

of the chromosomes can be followed with comparative ease all through the life-history.

The gamont nucleus gives rise to a small achromatic spindle, on to the equatorial plate of which three tiny chromosomes pass. These are formed from a little vesicular karyosome which lies near the spindle, and which is all that remains of the originally abundant chromatin of the nucleus. They are at first filamentar, but shorten and thicken until they become globular on the equatorial plate (see fig. 2, A). Each chromosome divides by constriction into two at the metaphase (fig. 2, B), and three daughter chromosomes pass to each pole of the spindle (fig. 2, C). Of the three chromosomes two are approximately equal in size and round in outline, while the third is slightly larger and ovoid.

In the second mitotic division the chromosomes are again present in the form of three globules. At the third, however, they are stumpy rods. But at the next division, and those immediately following it, they are filamentar. They split longitudinally at the metaphases (fig. 2, D) and pass as filaments to the poles of the spindles (fig. 2, E). A definite size-relation is usually visible—one chromosome being long, one somewhat shorter, and the third intermediate (*cf.* fig. 2, E).

During later divisions the nuclei become smaller, and travel to the periphery of the organism. At the same time the chromosomes become shorter and more stumpy (fig. 2, F). The nuclei multiply still further at the periphery. When they have become very numerous and small—immediately previous to gamete formation—the chromosomes are seen to be once more globular, and to divide by constriction (fig. 2, G). The gametes are now formed, and fuse in pairs.

When the nucleus of the zygote (sporoblast) is about to divide, its chromatin breaks up into numerous granules, which subsequently unite to form a spireme. The spireme is at first an open one, but it afterwards contracts into a somewhat tangled knot towards one side of the nucleus. The knot then opens out, and the spireme segments into six filamentar chromosomes (fig. 2, H). Of these two are long, two short, and two intermediate. At the division which now takes place these separate into two homologous sets of three each, which pass to the daughter nuclei (fig. 2, K). The latter rapidly divide twice in succession, thus giving rise to the eight sporozoite nuclei. Three chromosomes are visible at each of these divisions: but at the second they are short rods (fig. 2, M), and at the third globular (fig. 2, N).

It will thus be seen that the number of chromosomes in all the nuclear divisions of *D. schneideri* is, with one exception, three. At the first mitosis

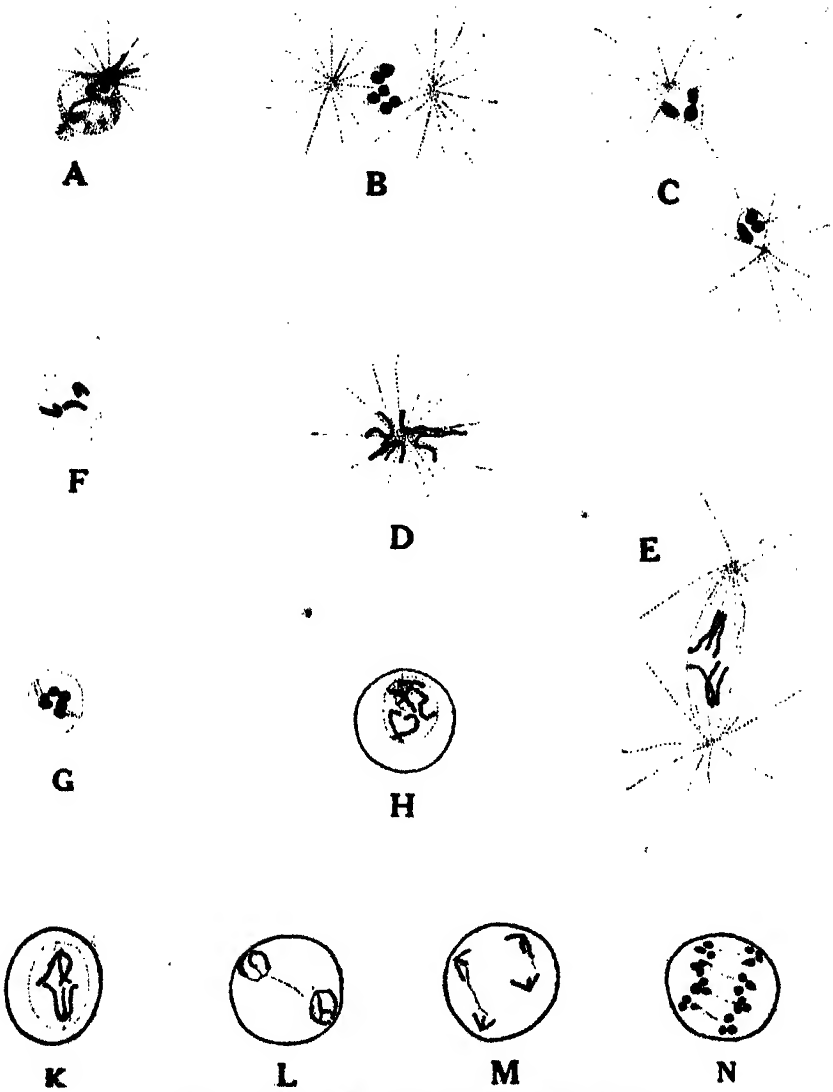


Fig. 2.—Chromosomes of *Diplocystis schneideri.*

The figures are tracings from drawings made with the camera lucida at a magnification of 2500 diameters. Only the chromosomes are exactly figured ; the other structures are semi-diagrammatic.

A. Early stage in first division of gamont nucleus. Chromosomes lying upon residual karyosome. The aster is also indicated. B. Metaphase of first division. C. Anaphase (late) of first division. D. Metaphase of later division, viewed from pole. E. Anaphase of later division. F. Equatorial plate stage of early peripheral division. G. Early anaphase of late peripheral division, immediately previous to gamete formation. H. Prophase (diakinesis) of first division in zygote (sporoblast). K. Anaphase of first division in zygote (spore). L. Early telophase of first division in spore. M. Anaphase groups at second division in spore. N. Anaphase groups at third division in spore.

in the spore, six chromosomes are formed by the segmentation of the spireme thread. These separate into two homologous groups of three each, which clearly represent the two sets of chromosomes derived from the two gamete nuclei which united to form the nucleus of the zygote. This first division in the spore—immediately following fertilization—must, therefore, be regarded as a reduction division.

The haploid number of chromosomes in *D. schneideri* is therefore three, and occurs in all the nuclear divisions of the gamont, and in all the spore divisions except the first. The diploid number is six, and is found at only one division in the whole life-cycle—the first sporal division, immediately following fertilization. Reduction thus occurs directly after fertilization, and not during gametogenesis.

Conclusions.

Although it is not yet possible to make any definite statement concerning the chromosome cycle of the Coccidia and Gregarines in general from the two individual instances which we have investigated, nevertheless we believe that certain justifiable conclusions can be drawn from the results of our work. We will briefly indicate what these are.

Both in *Aggregata* and in *Diplocystis* the chromosomes are so remarkably constant throughout the entire life-cycle that we believe this must be the case in other Coccidia and Gregarines also. The apparent exceptions, in which "amitotic" and "chromidial" nuclear divisions have been described, are probably in every instance referable to abnormal or degenerate conditions in the organisms studied, or to faulty cytological technique. We now know this to be true at least of *Aggregata* and *Diplocystis* and other Sporozoa which we have ourselves studied.

So far as we are aware, the chromosomes have not previously been exactly counted in any coccidian. "Reduction" has, however, been described in a number of forms (*cf.* Schaudinn, 1900; Siedlecki, 1898; etc.); but it is evident that this "reduction" has nothing whatever to do with reduction properly so called, *i.e.* with halving of the chromosome number. The "reduction" and "épuration nucléaire" described during gametogenesis are merely stages in the disintegration and elimination of the karyosome, and have nothing whatever to do with the formation of the gamete nuclei. These "reductions" have probably been so termed because they occur during gametogenesis; but in *Aggregata* at least—and probably in other coccidia—the chromosomes are present in the reduced number throughout this process.

In *Cyclospora* two "reduction-divisions" have been described (Schaudinn, 1902) in the maturation of the macrogamete. No chromosomes were observed,

however, and it seems to us probable that the phenomena described were misinterpeted. No "polar nuclei" like those described in *Cyclospora* are formed by *Aggregata*; and from the study of the chromosomes, it is clear that the occurrence of reduction-divisions at this stage is improbable, as the haploid number of chromosomes is already present.

In Gregarines the chromosomes have been more fully studied than in Coccidia. Their number has been counted in several forms, and it is significant that in some of these an odd number has been found. In addition to *D. schneideri* with three chromosomes, we may note *Echinomera hispida* with five (Schellack, 1907), *Nina gracilis* with five (Léger and Duboscq, 1909), and *Gregarina ovata* with three (Schellack, 1912). It is somewhat surprising that what is probably the correct explanation of this uneven number—namely, that it is the haploid number—has not previously been given. It has been suggested (Schellack, 1907; Léger and Duboscq, 1909) that the odd number is due to the presence of an "accessory" or "unpaired" chromosome—it being assumed that the odd number is the diploid number, and that reduction occurs in gametogenesis. We can find no good evidence to prove that a true "accessory" chromosome is really present in any of the organisms in which it has been described. The authors admit that the supposed "accessory" is characterized chiefly by its size—which is no criterion—though in *G. ovata* it is hardly, if at all, distinguishable from the other chromosomes (Schellack, 1912). It is also stated that this "accessory" or "axial" chromosome helps to form the karyosome of the resting nucleus; though in *G. ovata* a karyosome is not formed. We believe that the "axial" chromosome possesses no special significance, the not infrequent presence in Gregarines of an odd number of chromosomes being explained by the fact that it represents, as in *D. schneideri*, the reduced number.

There has already been a good deal of speculation concerning "reduction" in the Gregarines, though it rests upon a very small basis of fact. In *G. ovata*, Paehler (1904) and Schnitzler (1905) have described the formation of a "polar body" by an unequal division of the nucleus during the "maturation" of the gamete. Léger and Duboscq (1909) have described a similar "reduction" in *G. munieri*, though two "reduction-nuclei" are said to be formed in this case. Whatever the correct interpretation of these "polar nuclei" may be, it is important to notice that a halving of the chromosome number during their formation has in no case been demonstrated. Until this has been done, we cannot regard these observations as indicating that a real nuclear reduction occurs in this way during gametogenesis in the organisms studied. As, furthermore, the number of chromosomes present in these cases previous to the so-called "reduction" appears to be an odd one, it is difficult

to imagine how the halving of the number could occur. It appears to us highly probable that, as in *D. schneideri*, no reduction occurs at these stages in any of these organisms.

There are only two cases of alleged reduction during gametogenesis in Gregarines in which chromosome counts have been made. Recently Trégouboff (1914) claims to have established a reduction of this sort in *Stenophora juli*. The chromosomes are said to be reduced from four to two by an unequal nuclear division, which takes place in the macrogametes either before conjugation, or during conjugation, or after conjugation. It is impossible to examine Trégouboff's statements in detail here; but a careful investigation of his figures and descriptions leads us to believe that the phenomena observed have been misinterpreted. The evidence presented at least is far from establishing his contentions.

The only other case in which reduction is said to occur during gametogenesis, and in which the chromosomes have been counted, is that of *Monocystis rostrata*, described by Mulsow (1911). Here the chromosomes are stated to be reduced from eight to four at the nuclear division immediately preceding gamete formation. This account does not agree with those of others in which "polar nuclei" have been described, and is very different from what we have found in *Diplocystis*. From a careful examination of Mulsow's work we are at present inclined to believe that he was in reality dealing with two different species of *Monocystis*—one with eight chromosomes, the other with four; and that no reduction occurs during gametogenesis. Unfortunately, the division which we regard, on analogy with *D. schneideri*, as probably the reduction division—namely, the first nuclear division in the spore—is not properly described by Mulsow. The investigation of this division is, however, of the utmost importance for the establishment of his claims. If Mulsow's interpretations are correct, we shall be forced to conclude that the chromosome cycle of *M. rostrata* is fundamentally different from that of *D. schneideri*, and from that which we believe to occur in other Gregarines and in Coccidia.

We consider that neither of the above cases—*Stenophora* or *Monocystis*—is perfectly satisfactory, considered entirely on its own merits. And this is not to be wondered at if the chromosome cycle of the Gregarines in general is, as we believe, like that which we have found in *D. schneideri*. The chromosomes are here present in their haploid number during gametogenesis, and accordingly their reduction is not to be expected at this stage.

It is clear that the chromosome cycle of *Aggregata* agrees, in principle, with that of *Diplocystis*. In both forms the haploid number of chromosomes is found in every nucleus throughout the entire life-history, with the single

exception of the zygote nucleus. This is a diploid nucleus, containing two haploid groups of chromosomes derived from the two gamete nuclei which entered into its formation. The division of this nucleus is, in both *Aggregata* and *Diplocystis*, a reduction division, which reduces the diploid number to the haploid once more. From the point of view of the chromosomes, it is thus clear that the first division of the sporont nucleus of *Aggregata* is not homologous with the first division of the "sporont" (gamont) nucleus of *Diplocystis*, but with the first division of its spore nucleus—since these are the divisions during which the chromosome numbers are halved.

A chromosome cycle such as we have found in the two members of the Sporozoa here described has not, we believe, been previously demonstrated in any of the Protista. It has, however, been supposed—though not proved—to occur in some forms.

We regard the supposition that reduction occurs during gametogenesis in the Coccidia and Gregarines as an incorrect analogy drawn from other animals. The Coccidia and Gregarines are a very homogeneous group of organisms. And the chromosome cycle is, as a rule, so constant a character in any natural group of animals or plants, that we find every reason to believe at present that the chromosomes of the Sporozoa* generally will be found—when more fully studied—to behave like those of *Aggregata* and *Diplocystis*. There is at least, we believe, no evidence from other Coccidia, and but little from other Gregarines, which can be urged with any cogency against this view.

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* By "Sporozoa" we mean the so-called Telosporidia only. We regard the "Neosporidia" as totally unrelated organisms.

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Experiments on the Restoration of Paralysed Muscles by Means of Nerve Anastomosis. Part III.—*Anastomosis of the Brachial Plexus, with a Consideration of the Distribution of its Roots.**

By Robert Kennedy, M.A., D.Sc., M.D., St. Mungo Professor of Surgery in the University of Glasgow.

(Communicated by Prof. J. G. McKendrick, F.R.S. Received February 11, 1915.)

(Abstract.)

In two former communications† experiments on anastomosis of nerves as applied to the facial nerve in the dog and in the monkey and to the limb nerves in the dog were published. The series of experiments presented in this paper has reference to anastomosis as applied to the roots of the brachial plexus. In the brachial plexus, as compared with the nerves in the limb distal to the plexus, experiments of this nature require special consideration, as in the former the fibres to the same muscle or muscles usually pass in more than one root, and consequently any conclusions from the operation of anastomosis are liable to be fallacious, unless this is taken into account.

It is necessary, therefore, to consider in these experiments on anastomosis also the question of the distribution of the roots of the plexus.

In two of the experiments only a single root, namely, the fifth or the sixth cervical, was permanently cut off from its centres and attached to a neigh-

* The expense of this research has been defrayed by a Government Grant from the Royal Society.

† 'Phil. Trans.,' B, vol. 202, p. 93 (1911), and vol. 205, p. 27 (1914).

bouring root. In all the other experiments two or more than two roots were dealt with. In the case of the former the temporary loss of function of the limb was very early restored, namely, commencing in 10 and 36 days respectively, and being completed in a further period of 9 to 15 days respectively.

When two or more than two roots were divided more extensive and more abiding paralysis resulted, and the recovery commenced much later, namely, at periods ranging from 79 to 107 days, and reached its maximum in additional periods which varied from 32 to 39 days. The contrast was, therefore, very marked between the recoveries after section of less than two roots, and those following section of two or more than two roots, and led to the conclusion that in the former the cause of recovery was different from that in the latter.

In the experiments in which two or more roots were divided, some were anastomosed to the spinal accessory and others to another root or other roots of the plexus, and comparison is made of the recoveries in these two forms of experiments. Comparison is also made with the results obtained in the two previous communications, and a marked correspondence is found in the dates of recovery when that was clearly due to the substituted nerve.

The views of other authors as to the distribution of the roots of the plexus are discussed, and also the views with reference to the comparison between the plexus of Macacus and that of Man.

The author's observations of 38 human plexuses in which he has operated for injury to the roots of the plexus are then considered, and conclusions as to the distribution of the roots drawn from the distribution of the paralysis, the nature of the damage to the roots discovered at the operation, the effects of the excision of the damaged parts, and the results of stimulation of the fifth, sixth, and seventh cervical nerves during the operation. These results of stimulation are compared with the results of stimulation of the same roots made in Macacus.

The following are the general conclusions from the research:—

1. The brachial plexus of Macacus and that of Man are practically identical, at least as regards the fifth, sixth, and seventh cervical nerves, the variation being of the nature of a prefixture of the plexus in Man, but not to the extent of an entire root.

2. In Macacus section of the fifth nerve alone paralyses no muscle and limits no movement, although it may weaken some.

3. In Macacus section of the sixth and part of the seventh disturbs the function of the limb to an appreciable extent, but the disturbances can be compensated for and the movements regained, although probably with

diminished strength, without reunion of the roots and without aiding the recovery of function by anastomosis.

4. In Macacus section of both fifth and sixth nerves almost entirely or entirely paralyses the deltoid, but not entirely the flexors of the elbow or the supinators, but in Man section of these two roots not only completely paralyses the deltoid, but also the external rotators of the arm, the flexors of the arm to such an extent at least that they cannot produce flexion, and also the supinator brevis to such an extent that it cannot produce supination, and in some cases also paralyses the extensors in the forearm.

5. In Macacus, in order to paralyse completely not only the deltoid but also the flexors of the elbow and the supinator brevis, it is necessary to divide the fifth, sixth, and seventh nerves, as apparently more of the fibres to the flexors of the elbow pass in the seventh nerve than in the case of Man.

6. In Macacus the paralysis resulting from section of the fifth and sixth may be largely restored by anastomosis of the peripheral segments of the two roots to the seventh cervical nerve, or to the spinal accessory, and the resulting restoration of the muscles does not materially differ in date of onset, in progress, or in ultimate result in the two cases.

7. The time taken for restoration of function by means of anastomosis is approximately the same in the case of the brachial plexus in Macacus, and in the case of the limb nerves distal to the plexus in the Dog, and in the case of the facial nerve in the Dog and in Macacus.

Electrical Effects accompanying the Decomposition of Organic Compounds. II.—*Ionisation of the Gases produced during Fermentation.*

By M. C. Potter, Sc.D., M.A., Professor of Botany in the University of Durham.

(Communicated by Dr. A. D. Waller, F.R.S. Received February 26, 1915.)

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The Development of the Thymus, Epithelial Bodies, and Thyroid in the Marsupialia. Part I.—Trichosurus vulpecula.

By ELIZABETH A. FRASER, B.Sc. (Lond.), and Prof. J. P. HILL, F.R.S.

(Received March 10, 1915.)

(Abstract.)

In recent years much attention has been paid to the development of the thymus and thyroid glands in the higher Mammalia but no observations on the development of these structures are extant in the case of the Marsupialia. In this memoir the authors have attempted to fill this blank in our knowledge so far as the Diprotodont Trichosurus is concerned. They have had at their disposal an extensive material of that form comprising both uterine and fœtal specimens, grouped in 22 stages.

In the adult, the thymus is remarkable in that it consists of three pairs of glands, viz., a large paired superficial cervical thymus situated posteriorly to the submaxillary salivary glands and internally to the platysma on the ventral side of the anterior region of the neck, and two pairs of smaller thoracic or posterior cervical glands, situated the one behind the other, cranially to the pericardium and in relation to the corresponding common carotid arteries. These latter glands represent respectively paired thymus III and IV. They may remain separate or the two glands of the same side may unite with each other on one or both sides of the body.

The epithelial bodies (parathyroids so-called) comprise two pairs which are constantly present, viz., the primary epithelial bodies III and IV and in addition a variable number of accessory bodies, not necessarily paired, which are frequently found in connection both with the cervical and thoracic thymus glands. Epithelial body III lies adjacent to the fork of the common carotid artery, whilst epithelial body IV is usually situated in the proximity of thymus IV.

The thyroid is situated just caudally to the larynx and consists of two lateral lobes connected by a median bridge.

As concerns the development of the thymus, our observations show that the epithelial basis of the cervical gland is derived mainly at least from the ectodermal walls of the cranial portion of the closed-off cervical sinus, and to a smaller extent from the distal portion of the ductus ecto-entobranchialis II. The second gill-pouch in early stages is well developed and possesses an extensive area of fusion (closing membrane) with the second ectodermal groove. As development proceeds, however, the pouch becomes drawn out to

form an elongated tubular structure and its closing membrane becomes reduced in extent. At the same time, the portion of the ectodermal groove situated immediately above the sinus separates off from the ectoderm in continuity with the distal extremity of the second pouch. As the result, the latter comes to be connected with the sinus by a short distal segment, the ductus ecto-entobranchialis II above mentioned, formed partly of groove-ectoderm, partly of pouch-entoderm, the line of junction of the two parts being of the nature of an oblique overlap. The cervical sinus has meantime closed, partly, and indeed mainly, as the result of the growth forward of its dorso-caudal margins, partly as the result of the backgrowth of the hyoid arch, its original wide opening becoming reduced to a narrow slit-like passage, the cervical duct.

The primordium of the cervical thymus first appears in the form of a bulbous enlargement of the coalesced and thickened walls of the dorso-cranial angle of the sinus, which passes above into direct continuity with the ductus ecto-entobranchialis II, whilst below it extends down as far as the junction of pouch 3 with the sinus-ectoderm. As development proceeds, the primordium increases in size at the expense of the remainder of the cranial portion of the sinus, which completely closes up. It thus assumes the form of a solid pear-shaped mass composed of epithelial cells. It is connected for a time with the ectoderm of the cervical groove by a thin cellular cord but that eventually disappears and the primordium lies free in the mesoderm. It is thus evident that the epithelial basis of the cervical thymus is in greater part of ectodermal origin, but it seems probable that a small amount of entoderm derived from the ventral continuation of the second pouch is also included in it.

As concerns the differentiation of the third pouch, the connection of the latter with the pharynx gradually becomes narrowed, and at the same time its connection with the sinus-ectoderm becomes reduced to a thin cord (the ductus ecto-entobranchialis III), which eventually disappears. The dorsal part of the pouch retains its lumen, its ventral part on the contrary becomes solid and grows ventrally as a solid prolongation. Over the cranial wall of its dorsal portion the cells assume a regular columnar arrangement and stain deeply with eosin. This part constitutes the primordium of epithelial body III. Over the remainder of the pouch, including the entire caudal wall of its dorsal part and the solid ventral prolongation, the cells assume a less regular, looser arrangement and stain rather less deeply. This portion constitutes the primordium of thymus III. The two primordia so differentiated soon separate from each other. Epithelial body III, at first luminated, becomes solid and moves slightly forwards to take up its permanent position

near the bifurcation of the common carotid artery, whilst thymus III moves backwards.

The fourth gill-pouch is smaller than the third, but undergoes a corresponding development. Here, however, epithelial body IV is derived from the dorsal portion of the pouch, including both its cranial and caudal walls, whilst thymus IV takes origin from its ventral portion, including its solid ventral prolongation.

Trichosurus is the first mammal to be described in which a fully-developed thymus derived from the fourth gill-pouch is constantly present. Rudiments of a thymus IV have, however, been observed in a number of Eutheria (calf, cat, man, etc.), whilst thymus IV is regularly present in some reptiles, *e.g.*, Coluber and Tropidonotus. In respect of the constant presence of thymus IV, Trichosurus would appear to exhibit more primitive relations than any mammal hitherto investigated, whilst in respect of the mode of origin of thymus III, from the whole extent of the caudal wall of the pouch as well as from the ventral prolongation, Trichosurus would seem to furnish an example of the transitional stage between the Reptilian mode of thymus development (the thymus being an exclusively dorsal product of the gill-pouch) and the Eutherian mode (the thymus arising as a ventral product of the pouch).

The developmental history of the median thyroid primordium in Trichosurus does not differ essentially from that of other mammals. It gives origin without doubt to the main mass of the adult thyroid. It is probable, however, that the ultimo-branchial (post-branchial) bodies also contribute in some degree to the formation of the lateral thyroid lobes. The ultimo-branchial body appears in our earliest stage as a ventral prolongation of the small fifth gill-pouch. It very soon attains a considerable size and, after separating from the pharynx, it moves forwards and becomes closely connected with the dorso-medial surface of the corresponding lateral lobe of the thyroid. It then proliferates actively and gives off cellular sprouts which penetrate amongst, and become indistinguishable from, the cellular cords of the lateral lobe.

The Development of the Thymus, Epithelial Bodies, and Thyroid in the Marsupialia. Part II.—*Phascolarctos, Phascolomys and Perameles.*

By Elizabeth A. Fraser, B.Sc.

(Communicated by Prof. J. P. Hill, F.R.S. Received March 10, 1915.)

(Abstract.)

This communication is a further contribution to our knowledge of the origin and development of the thymus and thyroid glands in the Marsupialia and contains a short account of these glands in two of the Diprotodontia, viz. Phascolarctos and Phascolomys, and in one of the Polyprotodontia, viz. Perameles.

Phascolarctos and Phascolomys.

Phascolarctos and Phascolomys normally possess only the paired superficial cervical thymus gland; only in one fœtus of Phascolarctos out of nine examined was a representative of the paired thoracic thymus (thymus III) found. One pair of epithelial bodies, viz. epithelial body III, is always present, whilst a second pair, presumably epithelial body IV, although found in the wombat, may not always occur in the koala.

As in Trichosurus, the cervical thymus is mainly of ectodermal origin but entoderm to some extent takes part in its formation. In Phascolarctos, it arises from the coalesced walls of the dorsal portion of the cervical sinus opposite the third gill-pouch and extending down opposite to the connection of the fourth pouch with the sinus, the entodermal component being probably derived from both ductus ecto-entobranchialis III and IV. The third and fourth pouches have the same form as in Trichosurus but the fusion of ectoderm and entoderm, which constitutes the ductus ecto-entobranchialis III and IV, becomes very thick and solid, especially in the case of the ductus ecto-entobranchialis III, which passes into direct continuity with the primordium of the cervical thymus. Although the second pouch is long and narrow and an elongated ductus ecto-entobranchialis II is formed, the latter lies anterior to, and has no connection with, the thymus primordium. In Phascolomys, on the other hand, the primordium is derived from the ectoderm of the closed cranio-dorsal walls of the cervical sinus together with a certain amount of entoderm from the ductus ecto-entobranchialis II as in Trichosurus.

In Phascolomys, the whole of the third pouch apparently gives rise to epithelial body III and this is probably also true in the case of Phascolarctos

except for one fœtus, where a thymus III is developed. The second epithelial body, when present, owing to its posterior position and to its occasional occurrence close to the ultimobranchial body and the lateral lobe of the thyroid, may be regarded as having originated from the fourth pouch and therefore corresponds to epithelial body IV.

The thyroid, as in Trichosurus, develops chiefly from a median primordium. The ultimobranchial body here also becomes closely connected with the lateral lobes and gives off sprout-like processes which probably contribute to the formation of thyroid tissue. In Phascolarctos, the lateral lobes are remarkable in their tendency to be asymmetrical and to become divided up into several isolated portions.

In the possession of only one pair of superficial cervical thymus glands, Phascolarctos and Phascolomys exhibit a noteworthy parallelism with the mole, in which also the cervical thymus alone remains as the functional gland of the adult.

Perameles.

In Perameles, there is no superficial cervical thymus at any stage of development, but two pairs of well-developed thoracic glands are always present, as are also two epithelial bodies, viz., epithelial bodies III and IV.

The thymus is derived certainly from the ventral solid portion of pouches III and IV, the epithelial body arising from the dorsal portion of the pouch in each case, but whether the primordium of thymus III also takes origin, as in Trichosurus, from the caudal wall of the dorsal part of the pouch, could not be determined in the material available. The thymus glands attain a remarkably large size and may unite to form a single thymus on each side or may remain quite separated from each other.

The median thyroid primordium and the ultimobranchial body differentiate as in Trichosurus, but the evidence in favour of the participation of the ultimobranchial body in the formation of thyroid tissue is especially convincing in Perameles.

A Bacterial Test for Plant Food Accessories (Auximones).

By W. B. Bottomley, M.A., Professor of Botany, University of London, King's College.

(Communicated by Prof. F. W. Oliver, F.R.S. Received June 1, 1915.)

In a previous communication* attention was called to the significance of certain accessory food substances for normal plant growth. It was pointed out that the nutrition of a plant depends, not only upon the supply of mineral food constituents, but also upon the presence of certain accessory organic food substances, very small amounts of which are sufficient to satisfy the needs of the plant. These plant food accessories are analogous in some respects to the curative substances of beri-beri and scurvy which Suzuki calls "oryzanine," and for which Funk has suggested the name "vitamine," thinking they are of an amino nature. More recently Moore and his collaborators have applied the term "torulin" to the curative substance obtained from yeast.

Experiments in progress indicate that the plant food accessories resemble more closely the growth-stimulating food factors of Hopkins than the vitamines of Funk, and the term "auximone" (Gr. *αὔξιμος*, promoting growth) is suggested for them, being descriptive of their action rather than of their nature or composition, about which nothing definite is known.

Hitherto the only means of demonstrating the presence of these plant auximones has been their action on the higher plants. Unfortunately this is a long process and often unsatisfactory owing to the difficulty in maintaining constant environmental conditions during the comparatively long period of growth, and a more ready means of demonstrating their presence is desirable in order to facilitate further investigation of their constitution and properties.

The effect of the plant food accessories obtained from an alcoholic extract of bacterised peat on the growth and nitrogen fixation of *Azotobacter chroococcum*, described in a previous paper, suggested the possibility of a bacterial test for the more active fractions of the alcoholic extract. As experiments had shown that the fractions of this extract of bacterised peat obtained by means of phosphotungstic acid and by silver and baryta, according to the method already described, gave growth results with wheat plants, an investigation was made of the effect of these fractions on the growth of Azotobacter.

Eighteen flasks, each containing 100 c.c. of distilled water, 1 grm. mannite, 0·2 grm. K_2HPO_4, 0·02 grm. $MgSO_4$, and 0·2 grm. $CaCO_3$, were divided into

* 'Roy. Soc. Proc.,' B, vol. 88, pp. 237–247 (1914).

three series of six flasks each. The first series served to test the growth of the organism in normal culture. To each of the flasks of the second series was added the phosphotungstic fraction from 1 grm. of bacterised peat, and to the flasks of the third series the silver fraction from a similar quantity. The amount of dry substance thus added to each flask was 0·00017 grm. in the case of the phosphotungstic fraction, and 0·000035 grm. in the case of the silver fraction. Each flask was then inoculated with 1 c.c. of a uniform suspension of Azotobacter in distilled water, and two flasks from each series were sterilised to serve as controls. After incubation for 10 days at a temperature of 26° C., the contents of each flask were analysed by the Kjeldahl process for its nitrogen content. The results obtained are as follows:—

Table I.

Series.	Nitrogen-content.	Nitrogen fixation.	Mean nitrogen fixation.
	mgrm.	mgrm.	mgrm.
I. Normal mannite solution	1. Control 0·1		
	2. " 0·1		
	3. Culture 4·0	3·9	
	4. " 3·8	3·7	3·9
	5. " 4·0	3·9	
	6. " 4·1	4·0	
II. Normal mannite solution + phosphotungstic fraction from 1 grm. bacterised peat	1. Control 0·2		
	2. " 0·2		
	3. Culture 9·7	9·5	
	4. " 10·1	9·9	9·7
	5. " 10·0	9·8	
	6. " 9·9	9·7	
III. Normal mannite solution + silver fraction from 1 grm. bacterised peat	1. Control 0·2		
	2. " 0·2		
	3. Culture 10·3	10·1	
	4. " 10·5	10·3	10·4
	5. " 10·9	10·7	
	6. " 10·7	10·5	

Although these results were promising, the use of this method as a test for plant auximones was found to be unsuitable owing to the variability of the organism and the length of time required for incubation, and a shorter and more reliable method was desirable.

Preliminary experiments had already shown that the application of bacterised peat to the soil resulted in an increased production of nitrates, and an examination of the effect of these auximones on nitrification in the soil yielded further interesting results. Two equal quantities of soil weighing 2 lb. each were taken, and to one was added, in solution in distilled water, the phosphotungstic fraction of that weight of bacterised peat (30 grm.)

which, if incorporated with the soil, would have given a mixture of 10 parts of soil to one part of peat by volume, the proportion used in the preliminary experiments. The weight of solid matter thus introduced amounted to 0·051 grm. After small portions of each had been weighed out for analysis, the two samples of soil were put into wide-mouthed glass bottles, loosely corked, and kept at laboratory temperature for some weeks, the bottles being well shaken daily to ensure aëration, and distilled water added when necessary, to maintain a uniform moisture-content. Small samples were taken from time to time, and their nitrate-content determined by the phenol-sulphonic acid method, with the following results :—

Table II.

	Nitric nitrogen in parts per million on—				
	April 6.	April 20.	April 30.	May 12.	May 26.
Soil	11	78	95	228	316
Soil + auximone	14	153	305	471	662

These results suggested that liquid cultures of the nitrifying organisms might furnish a test for plant auximones. A culture was therefore obtained by placing 10 grm. of garden soil in a flask containing 100 c.c. tap-water, 0·1 grm. $(NH_4)_2SO_4$, 0·1 grm. K_2HPO_4 and 0·2 grm. $MgCO_3$ (Winogradsky's medium), and incubating for seven days at 26° C., at the end of which period the liquid showed a strong reaction for nitrate. Sub-cultures were then made from this liquid into fresh nitrifying solutions, and, after a further week's incubation, a second sub-culture was made, which was used for testing the effect of the auximone.

A series of eighteen flasks was then prepared, six containing normal nitrifying culture solution, six the normal solution plus phosphotungstic fraction from 1 grm. of bacterised peat, and six the normal solution with the addition of the silver fraction. All were inoculated from the second sub-culture of nitrifying organisms and incubated at 26° C. At the end of 48 hours all the flasks containing auximone showed a thick scum on the surface of the liquid, and when examined at the end of six days were found to contain no trace of nitrate, while in those flasks without auximone, where no scum had developed, nitrification had proceeded normally. Some contamination of the medium was suspected, and the work was repeated, great care being taken with the sterilisation of flasks and media. Again a scum appeared in all the liquids containing auximone, and a third experiment yielded similar results.

The constant formation of this scum whenever the auximone was added to the crude nitrifying culture from soil suggested the possibility that either the scum-forming organisms were introduced with the auximone, or the scum formation might be used as a specific test for auximones. To test this a sub-culture was made from an original soil culture and incubated for four days. No scum formed. This was then divided into two portions, one of which was autoclaved at 140° C. for half an hour, after which the phosphotungstic fraction from 1 grm. of bacterised peat was added to each, and both were re-incubated for three days at 26° C. A thick scum formed on the unsterilised liquid, but no trace appeared on the sterilised one, thus showing that the scum organisms are present in the soil culture, and the formation of the scum is due to the presence of the auximone.

An examination of the scum shows that it consists of two predominant kinds of organisms: a thin beaded-rod form and a spindle-shaped form. The nature of the scum depends upon the relative proportion of these two organisms, being crinkled and gelatinous when the beaded forms predominate, and smooth and brittle when the spindle forms are in a majority. By continuous plating out pure colonies of each of these forms were obtained, but when grown separately in nitrifying solution plus auximone the characteristic scum never appeared. Further investigations are in progress as to the identity and nature of these organisms.

The soil from which the scum-forming organisms were first obtained was a rich garden soil from Kew, and an examination of other soils was made in order to determine whether the organisms are of fairly constant occurrence or whether they are restricted to certain localities. Upwards of a dozen samples of soils, including loams, clays and gravel, from various places were tested, and all were found to yield the characteristic growth in the nitrifying solution with the addition of auximone. The rapidity of formation of the scum, however, varied considerably, the best and most rapid growth being obtained from a sample of new loam from a virgin (uncultivated) field; a very good growth from some old potting mixture; and a very slow and poor growth from soil from a bed of leguminous plants.

Although the organisms are thus found to be widely distributed and easily obtainable, it became necessary for experimental purposes to obtain a uniform stock from which a good growth could be readily obtained. Some soil was therefore sterilised, put aside for a week, and then saturated with a suspension of the scum-forming organisms. It was allowed to dry down at room temperature under sterile conditions, and stored in a bottle. This stock can be depended upon to yield a good growth of scum in from two to three days in the presence of auximones.

The scum is most readily obtained by placing about 10 grm. of soil in a nitrifying solution and incubating it for two days before adding the auximone. The first trace of scum appears in 24 hours after this addition, and increases as incubation is continued, until at the end of four to six days it becomes so thick that it sinks to the bottom of the flask, and no second scum is formed. Sub-cultures from this growth are used for test purposes. It is found, however, that successive sub-culturing from the original scum rapidly produces an alteration in the nature of the growth, very little scum being formed, and the liquid becoming turbid and bright yellow in colour. Hence the necessity for obtaining a fresh scum from the prepared soil for each new set of experiments.

In order to test whether these organisms are able to indicate the relative quantity of plant auximone present, six series of three flasks each were arranged as follows:

Series.	Nos.						
A	1–3	Contained 100 c.c. normal culture solution.					
B	4–6	"	"	"	+4·2	parts per million silver fraction from bacterised peat.	
C	7–9	"	"	"	+2·1	"	"
D	10–12	"	"	"	+0·35	"	"
E	13–15	"	"	"	+0·07	"	"
F	16–18	"	"	"	+0·007	"	"

All were inoculated with the scum-forming organisms, and incubated at 26° C. After 36 hours, series A showed no trace of scum, B an extremely thick one, C a thick one, D a moderate growth, E a fair growth, but not nearly so good as D, and F no appreciable growth; hence the rate of growth and thickness of scum show a progressive increase with the quantity of auximone present above a certain minimum, which in this case was the extract from 0·2 grm. of bacterised peat.

The fact that this minimum amount, which represents only one part of the dry silver fraction in sixteen millions of culture solution, gives a formation of scum indicates the sensitiveness of the organisms to a very minute trace of auximone.

As there was a possibility of other substances present in the phosphotungstic and silver fractions being concerned in the scum formation, an investigation was made of the effect of the presence of certain organic substances in the nitrifying solution. The usual standard employed in tests with bacterised peat extract has been the phosphotungstic fraction from 1 grm. of bacterised peat per 100 c.c. of culture solution, which represents a solution of seventeen parts of dry substance per million of liquid.

Accordingly, nitrifying solutions containing seventeen parts per million of sucrose, maltose, asparagine, peptone, leucine, tyrosine, and hordein respectively, were inoculated with the organisms, but no growth occurred after four days. Fresh liquids, containing twenty, forty, and sixty parts per million of each of these substances were then tested, with negative results; and not until the proportions had been increased to two hundred parts per million in each case was any growth apparent. Even then the characteristic scum was not obtained, the whole medium becoming uniformly cloudy. This shows that the scum formation is due to the specific action of auximones.

The next step was to determine if the presence of the accessory substances concerned with animal nutrition would induce scum formation. These substances have been obtained from various seeds and yeast. For this experiment the seeds employed were wheat, maize, and peas. They were first soaked in water for 24 hours, then dried and left at room temperature for two days, when their radicles were from ½ to 1 inch in length. They were then ground up in a mortar, and a phosphotungstic fraction obtained of these, of similar quantities of the dry seeds, and of yeast, in the same way as described for bacterised peat. The extract of 1 grm. of each of the seeds and of yeast was used for testing, and no scum could be obtained with the phosphotungstic fraction of dry maize and dry peas. A fair scum, however, was obtained in from two to three days with the fractions from yeast, germinated wheat, peas, and maize, and a thin scum from dry wheat. In the case of wheat, the seeds are invested with a pericarp, and it was from this region of rice grains that an animal food accessory was first obtained. It is thus evident that the scum-forming organisms are able to serve as a qualitative test for food accessories in general.

Having thus obtained an indicator for auximones, it becomes a comparatively simple matter to examine other materials for their presence. Since bacterised peat, in which auximones are relatively abundant, is obtained by bacterial action upon raw peat, it was decided to investigate other samples of decomposing organic matter for these substances. Quantities of fresh stable manure, and also of a well-rotted two-year-old manure, were procured and fractionated in the usual manner. Portions of these fractions, corresponding to 1, 2, 4, 6, 8, 10, 20, 40, and 50 grm. respectively, of both fresh and rotted manures were added to nitrifying solutions, three flasks being used for the investigation of each of the separate portions. After inoculation with the scum-forming organism, the whole series of flasks was incubated for four days when the first indication of a scum appeared in the liquids containing the fraction from 10 grm. of rotted manure, and in those which had received the

extract from 50 grm. of fresh manure. It would thus appear that the quantity of auximone present increases with the progressive decomposition of the organic matter of the manure; although the relatively small amount even in the two-year-old rotted manure is apparent, when it is stated that a better formation of scum was obtained with the fraction from $\frac{1}{4}$ grm. of bacterised peat than with 10 grm. of the manure.

An unexpected source of plant auximones has been discovered in the root nodules of leguminous plants. A quantity of root nodules from bean plants were collected, and the phosphotungstic fraction obtained from them in the usual way. The roots which bore the nodules were also extracted separately. On investigation it was found that a very thin film was obtained when nitrifying solutions containing the phosphotungstic fractions of $\frac{1}{40}$ grm. of roots and nodules respectively were inoculated with the scum-forming organism. Liquids containing $\frac{1}{20}$ grm. gave a fair growth, and those containing $\frac{1}{10}$ grm. a good growth, the extract from nodules giving a slightly better growth than that from the roots in each case. A similar fraction was obtained of the roots of beans which had been grown in sterilised sand and which had formed no nodules, and no growth at all could be obtained upon nitrifying solutions to which had been added the extract from $\frac{1}{10}$ grm. of such roots.

Some further interesting points may be noted in connection with this research. The organisms which form the scum require no organic carbon for their growth, and are similar to the nitrifying organisms and sulphur and iron bacteria in that they can assimilate atmospheric carbon dioxide by the process of chemosynthesis. Further they cannot live on nitrates, but must obtain their nitrogen from an ammonium salt.

The plant auximones so far investigated differ in one important respect from those concerned with animal nutrition in that they are not destroyed by heating. A phosphotungstic extract from bacterised peat gave a thick scum after being heated in an autoclave at 134° C. for half an hour.

Hitherto lack of knowledge of the nature of plant auximones has retarded research, but it is hoped that, just as the investigation of "deficiency" diseases was promoted by Eykman's* production of polyneuritis in birds, and the inducement of scurvy in guinea-pigs by Fürst†, the discovery of a bacterial test will facilitate an examination of the occurrence, nature, and composition of plant auximones.

* Eykman, 'Virchow's Arch.,' vol. 148, p. 523 (1897).

† Fürst, 'Verh. des 6 Nord. Kongress f. Inn. Med.,' p. 342 (1909).

The Inheritance of Colour in the Stick-Insect, Carausius morosus.

By E. W. MacBride, F.R.S., and Miss A. Jackson.

(Received June 1, 1915.)

[Plates 4 and 5.]

The stick-insect, *Carausius* (*Dixippus*) *morosus*, is an inhabitant of Southern India. The genus *Carausius* is closely allied to the genus *Dixippus*, from which the principal feature which separates it is a keeled sternum on the mesothoracic segment in *Carausius*, and the absence of such a keel in *Dixippus*. In *Carausius morosus* this keel is obscure, and Wattenwyl and Redtenbacher (1908), whose classification we follow, include this species in the genus *Carausius* on general grounds. Other authors have assigned the species to *Dixippus*, whence it comes that in the literature the species is frequently referred to as *Dixippus morosus*.

It has been known for some time that this insect exhibited marked colour varieties, and it occurred to us that it would be an interesting research to determine the laws governing the inheritance of these variations. Our material consisted of a batch of eggs received from India in 1911. A preliminary research by the senior author in 1912 yielded such curious results that it was determined to take up the investigation in detail, and to this subject the junior author has devoted the last two years.

Before detailing the results at which we have arrived, it will be desirable to give an outline of our previous knowledge of the subject. The life-history, habits, and principal colour variations of *Carausius morosus* were dealt with in a paper by Meissner (1909), who received a batch of 300 larvæ which had just hatched, and reared them until maturity was attained. It is almost certain, however, that in supposing his larvæ to be just hatched Meissner was mistaken, and that in reality they had completed their first moult when he received them, for he asserts that egg-laying begins after six moults, whereas according to our observations seven moults are passed through before sexual maturity is attained.

All his specimens were green when he received them, but out of 41 adults successfully reared 17 were green and 24 brown. He states that when the brown insects are kept in the dark between the fourth and fifth moult the darkness of their colour becomes intensified.

De Sinety, in a paper dealing with the biology and anatomy of the Phasmidæ (1901), includes *Carausius morosus* amongst the species he investigated. He reared both males and females, and commits himself to the

extraordinary statement that the male proceeds from a fertilised egg, whereas the female is parthenogenetically produced. He kept a dozen larvæ in darkness from hatching, and found that they began to turn dark about the third moult.

Schleip (1911) deals with the colour varieties of the adult insect and with the changes in colour produced by variation in light. He classifies the adult varieties of coloration into four types—(*a*) green, (*b*) green with yellowish spots, (*c*) yellowish brown, and (*d*) brown. Of these types (*a*) included half the individuals, (*b*) about a quarter, (*c*) about 5 per cent., and (*d*) about a quarter. Examination of the ectoderm reveals the fact that three distinct pigments are concerned in producing the colour, viz., a green pigment distributed in small grains throughout the cytoplasm of the cells, a brown pigment distributed in large round grains in the base of each cell, and a yellowish-red pigment distributed in angular masses near the centre of the cell in the vicinity of the nucleus. In the green variety of insect there is always a certain amount of brown pigment present. In the reddish and brown varieties the green pigment is absent and is replaced by greyish dots. Schleip maintains that the brown varieties become darker when kept in the dark—contrary to the general rule for such changes, because melanic pigmentation of all kinds when subject to change usually becomes lighter in the dark. This increase in the darkness of brown individuals is due, according to him, to the streaming of the brown pigment over the cell. The brown pigment granules leave their basal position and stream over the cell.

We may say at once that we have not been able to confirm these observations of Schleip; but whether they are well founded or not they have no bearing on the question of there being at least two well marked categories of insects so far as relates to colour, viz., those with green pigment and those without. It is obvious also that in the green category belong all those subdivisions which can be described as olive-green and green with spots.

Methods Employed.

Our preliminary experiments showed us that the insects reared from the eggs with which we began our research were in all cases parthenogenetic females. These when mature acquire a bright patch of scarlet coloration on the inner sides of the femora of the forelegs and then begin to lay eggs. Part of the original batch of eggs was given to the Insect Department of the Natural History Museum, and amongst the insects raised by them one male appeared. As the male is much smaller than the female and liable to be confounded with an immature female, this discovery led us to discard the results of our initial experiments, and to take especial precautions against

the accidental fertilisation of females. We raised altogether after this several thousand insects, and amongst these six males and one gynandromorph appeared, which were at once detected and removed. Our results are, therefore, concerned with parthenogenetic inheritance.

The insects were kept in glass cylinders covered at the top with muslin, and were fed on branches of privet, the ends of which were immersed in small tubes of water. In this way the food plant was kept fresh until all the leaves had been devoured. The eggs, which resemble small round seeds, drop to the bottom of the jar ; they can be preserved dry in pill boxes for months without injury: in fact, they normally pass through a resting period of four or five months. Later, however, we found that it was advisable to spray them once a week—for in this way a larger proportion of larvæ hatched.

During the time of hatching the atmosphere in which the insects live must be kept thoroughly moist. There is considerable mortality during the hatching—many insects die with the hinder end still caught in the egg shell. By keeping the atmosphere moist this mortality is very much reduced.

Females were isolated from the fourth or fifth moult and reared to maturity. Their eggs were then collected and raised to maturity, and the proportion of colour varieties ascertained. Although the certainty of non-fertilisation only extends back one generation, the accordance of the results obtained with those obtained before the presence of males was suspected not only confirms our belief in the reliability of the results which we have obtained but tends to prove the extreme improbability that the accidental fertilisation of a female vitiated our earlier results.

Colour Varieties of the Adult Female.

No less than 10 grades of colour can be distinguished in the adult female, viz. (1) green, (2) olive-green, (3) green suffused with brown, (4) green dappled with brown, (5) yellow, (6) yellowish brown, (7) red brown, (8) hazel brown, (9) dark brown, (10) brownish black. Of these Nos. 1–4 are to be reckoned as green. We have already seen that all varieties of green evidently represent the modification of this tint by increasing amounts of brown pigment. Nos. 5, 6, and 7 are characterised by the prominence of the yellowish-red pigment, whilst Nos. 8, 9, and 10 belong evidently to the brown type and differ only in the intensity of their pigmentation. Six of the varieties are shown in fig. 1. In this figure also the scarlet coloration denoting sexual maturity is seen on the inner surfaces of the forelegs.

The Adult Male.

Amongst 3000 insects bred seven males were found.

The male insect is about the size which the female attained at the fifth moult. Its sexual ripeness is indicated by a blush of scarlet on the under surface of the meso- and meta-thorax; there are also two narrow lines of scarlet on the dorsal surface of the metathorax. The ground colour of the specimen shown in fig. 2 is greyish olive. Another male had a ground colour of greenish yellow and the abdomen was suffused with pure yellow. In such a small collection it is naturally impossible to say how many colour varieties of male exist, but obviously there must be at least two. The abdomen has the characteristic shape found in the males of Orthoptera.

The Just-hatched Insect.

So far as our observations go, all insects when they emerge from the egg are alike, whatever colour they may afterwards assume; Meissner, it will be remembered, described them as green, and de Sinety as being "d'une couleur fuligineuse." Neither description is correct. The insect on hatching has a definite colour pattern. The mid-dorsal line is occupied by a fine green band, very narrow on the head, but widening as it proceeds backwards until it is fairly wide in the region of the abdominal segments and a constriction between two adjacent segments is observable, so that the patches of greenish-yellow copy in shape the underlying chambers of the heart. At the sides of this median band are two bands of brown, very wide in the region of the head but narrowing as they proceed backwards until they become linear in the abdomen. These bands are bounded laterally by pale green streaks. Then on the lateral edges of the segments there is another brown band, broad on the head region where it crosses the eye and becoming narrower as it proceeds backwards (see fig. 4). The ventral surface is yellowish-green, becoming more and more mottled with brown as one proceeds backwards till in the last three segments of the abdomen the green has disappeared.

Changes of Colour as Development Proceeds.

If the insect be one of the pure green variety the brown bands become more and more broken up into mottling, and often when the first moult has taken place the insect is nearly pure green, although the brown band on each side of the head could still be made out. No abrupt change of colour takes place at the moult itself, because, as we have already seen, the pigment granules are situated in the ectodermal cells: the cuticle is practically colourless. In one special instance noted, an insect hatched on May 7 as the

first instar. The first moult leading to the second instar occurred on May 19. The only traces of brown left were the brown band on the side of the head and very faint traces (discernible only under close examination) of the other bands. The second moult leading to the third instar occurred on June 12. After it the same traces of brown were discernible as in the second instar, but after the third moult on July 1 all traces of brown except a few tiny dots on the dorsal surface of the thorax and of the abdominal segments had disappeared. After the fourth moult on July 24 nothing but green was discernible. This continued after the fifth moult on August 22 except for the appearance of a slight tinge of red on the anterior edge of the femora of each of the first pair of legs. After the sixth moult on September 14 the red tinge had developed into a band of pale pink. After the seventh and last moult on October 18 the band of pink had become bright vermilion, and the eighth or adult instar had been reached. A month later egg laying began, and on an average one egg was laid per day during the hours of darkness.

Table of Length of the Various Instars.
(Compiled from 12 insects.)

	mm.
1st instar	11–14
2nd "	14–19
3rd "	20–25
4th "	27–31
5th "	33–40
6th "	45–52
7th "	59–67
8th " (adult)	70–81

Duration of Instars.
(Compiled from 8 insects kept at a temperature of 40°–60° F.)

	days.
1st instar	13–19
2nd "	14–22
3rd "	20–25
4th "	19–34
5th "	24–31
6th "	25–32
7th "	35–47

As we have stated below all insects are hatched alike in colour pattern, and the change to pure green takes place after the first moult and is complete after the third. After the first moult also a change to brown may occur as shown in fig. 5.

An apparently pure green may become olive-green in the fourth instar, and may remain so, or it may become light brown and, finally, dark brown, or from olive-green it may become reddish-brown.

Yellowish-browns appear in the fourth instar, and the colour may deepen into hazel. A very curious and exceptional change is that a red-brown appearing in the second instar may develop into green suffused with brown.

Proportions of Colours—Varieties in the Offspring.

In October, 1912, 30 adult females were isolated in glass jars, and allowed to lay eggs. The eggs hatched in April, 1913, and the emerging insects were reared to maturity, and the proportion of colour varieties produced was ascertained.

The mortality amongst these insects was very high. Out of a total of 174 insects reared 147 were green and 27 brown, giving a ratio of between 5 and 6 green to 1 brown. The most interesting result was that the colour of the parent made no difference in the proportions of colour variations found in the offspring. To give instances: Out of 10 insects, the offspring of a brown female whose mother was also brown, 8 were green and 2 brown. Again, out of 14 insects, the offspring of a green female whose mother was green, 11 were green and 3 brown. From a green parent whose mother was brown, 13 insects were reared, and of these 11 were green and 2 brown.

This result was sufficiently unexpected to whet the desire for more detailed knowledge. It is open to two objections. First, the proportion of larvæ which survived is small, and, secondly, from some of the original batch of eggs from which the material was derived one male was reared. It was, therefore, determined to repeat the experiments, taking precautions to keep the atmosphere warm and moist during hatching.

In August, 1913, 40 females in the fourth and fifth instar were isolated, and placed each one in a separate jar on a food plant. These females became adult in September, and laid eggs in October and November, and throughout the winter. One insect continued to lay till September, 1914, and laid in all 500 eggs.

The eggs produced by each specimen were counted and placed in a pill box. They were sprayed once or twice a week in order to prevent them becoming too dry. The eggs began to hatch in April, 1914. As hatching was spread over a considerable time, maturity was reached at very different times by the different families, and so from time to time counts were made and the adults removed. The first of these counts was in September, 1914, the

last in April, 1915. In all, over 3000 insects were reared. About half the families were reserved for experiments on the effect of reduced light and darkness (*v. infra*); the remaining 1676 insects were reared under normal conditions, and of these 1612 were green and 62 brown, whilst 2 were classified as yellow. Leaving these two out of account, the proportion of brown to green is not quite 4 per cent. or 1 in 25. If we examine the case of individual insects this conclusion is amply borne out. Of 91 young of a green insect whose mother was brown 89 were green and 2 brown. Of 105 offspring of a brown insect whose mother was green 103 were green and 2 brown. It must be remarked that the mortality in these later experiments was very much reduced. Whereas in the 1913 experiments from 2056 eggs 174 insects were reared, in 1914 and 1915 from 4746 eggs 1676 insects were raised. The proportion of browns amongst adults was in the first case 15·5 per cent., in the second case 3·6 per cent. As the mortality is reduced therefore the proportion of browns diminishes.

It is obvious that we have to do with a type of segregation of varieties, but a type which has not hitherto been described. In the first place the insects are almost certainly the offspring of three or four generations of parthenogenetic development. If the process of segregation were the result of an originally hybrid constitution of the females which laid the eggs which we received from India, then, even if an initial segregation had taken place, since the only reproduction is parthenogenetic this segregation ought to cease, after the first generation, since no crossing amongst the F_1 generation takes place. Then the proportions of brown to green do not bear any relation to any Mendelian ratio, and a brown parent produces just the same proportion of browns to greens as a green parent. At the same time, whilst the factor for brown is present in all insects, the factor for green is definitely eliminated in a certain proportion as growth proceeds.

It is an obvious reflection that possibly the production of a brown rather than a green adult may be due to a colour reaction—those larvæ, it might be supposed, which pass a critical period of their growth in a shaded environment turn brown, whilst the rest exposed to full illumination turn green.

In order to test this hypothesis the experiment was made of taking the eggs laid by a certain number of insects and dividing the progeny of each into two equal lots, one lot being reared as in other experiments in glass jars closed at the top by muslin, the other in glass jars of equal size closed at the top by two layers of muslin and pasted round the sides by white paper so that only light of reduced intensity could reach them. The two lots were compared in order to see if a greater proportion of browns were produced by the insect which were exposed to diffused light.

The result of this experiment was to show that on the whole fewer browns were produced by insects subjected to diminished light than by those exposed to intense light. To give examples: Of 384 eggs, the progeny of one female, 192 were subjected to bright light and 192 to diffuse light. Of the first lot 55 were reared, including 52 greens and 3 browns: of the second lot 64 were reared, viz., 63 greens and 1 brown. In another case 222 eggs, the progeny of one female, were taken, of these 111 were exposed to bright light and 111 to diffused light. Of the first lot 75 were reared, 72 green and 3 brown—of the second lot 87 were raised, all green.

An attempt was then made to rear a certain number of insects in complete darkness, making use of an extremely ingenious piece of apparatus devised for us by Prof. Lefroy. This consisted of a light-tight box of stout deal divided into an inner and an outer compartment, the whole being painted black. The two compartments communicated with one another by means of a zinc shutter over which was a weighted black curtain. The outer compartment was closed by a wooden frame over which a black curtain was stretched. The frame worked on a hinge and could be raised. In the curtain there were a pair of holes to which black sleeves were attached. These sleeves admitted the arms of an operator, round whose wrists they were tightly secured by elastic. The just-hatched insects were placed on a privet plant in the inner compartment which was watered through an external funnel communicating with the interior by a rubber tube controlled by a tap placed internally. When it was necessary to change the plant the outer compartment was opened and the new plant was placed therein. Then, the outer curtain being down, the arms of the operator were introduced through the sleeves and the wooden frame raised. Then the zinc shutter of the inner compartment was raised and the insects were shaken off the old plant, which was then brought into the outer compartment and replaced by the new one and the zinc shutter closed. When by feeling it was determined that the insects had become adult the case was opened.

Three such cases were made. In the first 50 insects were placed immediately after hatching, of which only four survived, and of these one was green and three brown. In the second 50 insects were also placed, but this case was opened from time to time for a minute or two to inspect the insects. Eight insects became adult, and all were green. In the third case an equal number of insects were placed, these were fed on germinating beans, which, growing in the dark, produced only yellowish leaves devoid of chlorophyll. Only one insect survived, and it was vividly green, as if it had been produced under normal conditions. From these experiments it is to be

concluded that green insects can be produced in perfect darkness and in the entire absence of chlorophyll.

The experiment was also tried of keeping the insects in continuous light day and night. The light was supplied by a 16-candle-power electric bulb. Twelve just-hatched insects were selected for this experiment. Instead of resting by day and feeding by night they fed by night as well as by day, and when resting did not assume the protective position. The insects were kept under perpetual light conditions from May 20 till February, with the exception of a fortnight in August. They became adult in September, and were fully six weeks in advance in development of other insects brought up under normal conditions. All were green.

General Conclusions.

The series of unexpected facts brought to light by this investigation do not fit in easily with any current theories. They lead to the following conclusions:—

(1) All stick-insects of the species *Carausius morosus* are born alike. All have a definite colour pattern of green and brown pigments.

(2) As growth proceeds, in the vast majority of the cases the green pigment overpowers the brown so that the pattern disappears, and the insect appears to the unaided eye a pure green. According to Schleip some traces of brown pigment can always be detected in the cells of the ectoderm of the greenest insects.

(3) In about 3 per cent. of the cases the brown pigment increases during growth and prevents any trace of green being seen, and in these cases, according to Schleip, no green pigment is visible in the ectodermal cells even on microscopic examination.

(4) In a few cases where the green pigment becomes predominant the brown pigment remains in sufficient quantity so as to affect the colour, and to give rise to the varieties known as green suffused with brown, green with brown spots, etc.

(5) In a still smaller number of cases the yellowish red pigment, which appears always to be present to some extent, increases so as to give the adult insect a yellowish or reddish brown colour.

(6) The proportion of insects which acquire a predominantly green or a predominantly brown colour amongst the offspring is not influenced by the colour of the mother.

(7) Pure green insects can be raised from larvæ exposed to complete darkness from hatching.

(8) Rearing insects in reduced light does not increase the proportion of

browns: there is no evidence in favour of the view that an insect becomes brown if reared in the shade.

(9) In a general way the production of a small proportion of browns is a help to the concealment of the animal under normal conditions, since such specimens resemble dried twigs.

(10) Males are produced from the eggs of unfertilised females, but are extremely rare.

In conclusion we should like to express our deep obligation to Prof. Lefroy for his invaluable help, both in giving advice as to methods of rearing and in devising apparatus.

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EXPLANATION OF PLATES.

Fig. 1.—Six specimens of adult female seen from the side, to illustrate some of the variations in colour. Reduced to two-thirds of the actual length.

A. Light brown or hazel variety.
B. Dark brown variety.
C. Yellow variety.
D. Red brown variety.
E. Pure green variety.
F. Olive green variety.

Fig. 2.—The adult male.

A. From the side.
B. From beneath, showing the rosy flush which indicates sexual maturity. Reduced to two-thirds of the actual length.

Fig. 3.—The just-hatched insect emerging from the egg. Slightly enlarged.

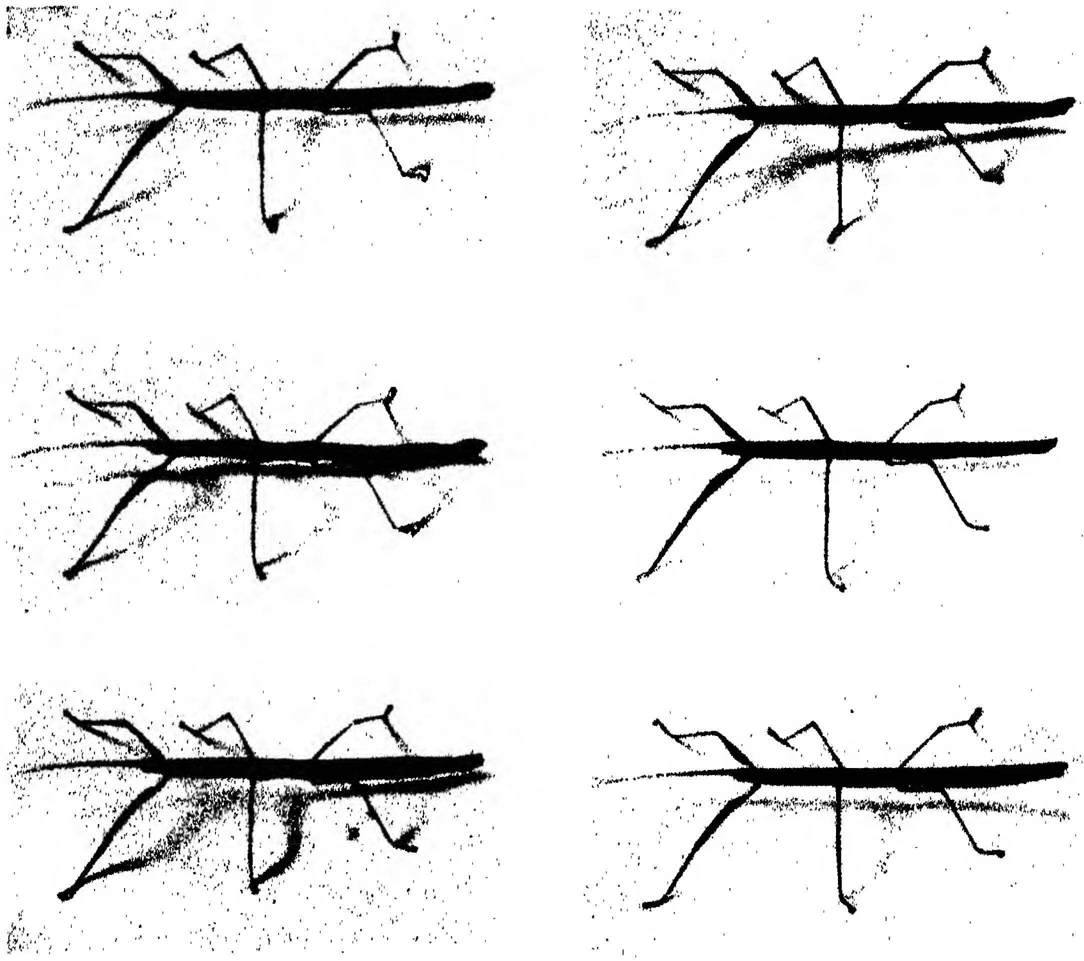
Fig. 4.—The just-hatched insect. Enlarged five diameters.

A. Seen from above.
B. Seen from the side.

Fig. 5.—Two insects in the second instar. Magnified two and a half diameters.

A. An insect which has turned brown, seen from beneath.
B. The same insect seen from above.
C. An insect which has turned green, seen from beneath.
D. The same insect seen from above.

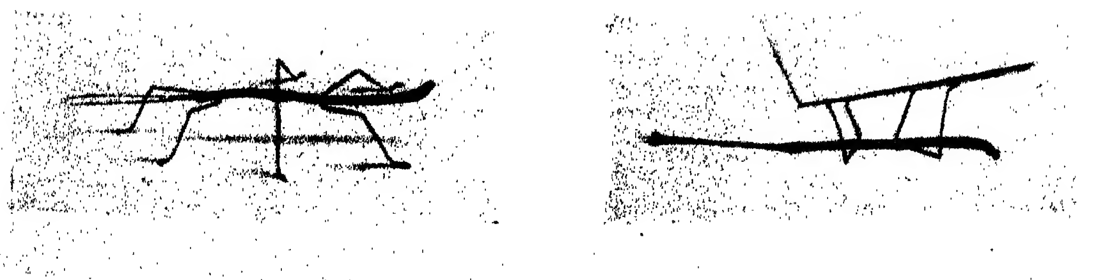
FIG. 1.



A B C D E F

FIG. 2.

A. B.



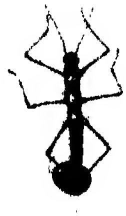


Fig. 3.

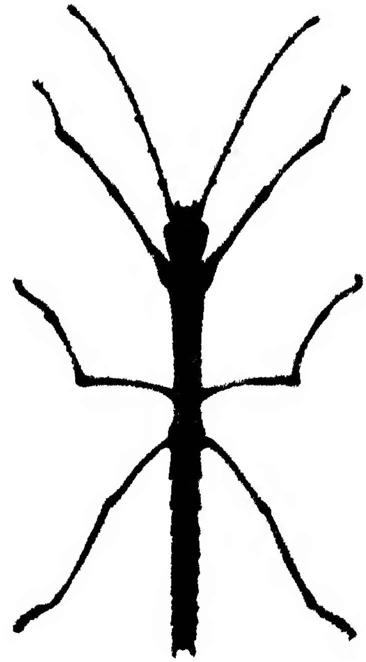


Fig. 4A

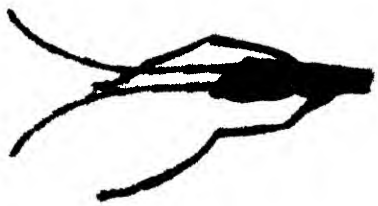


Fig. 4B.

Fig. 5A.

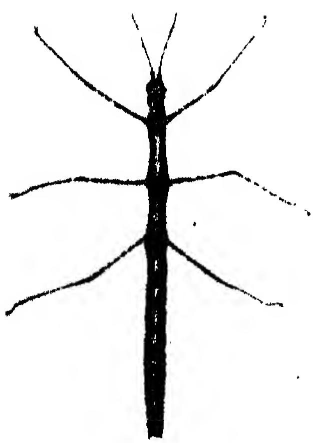
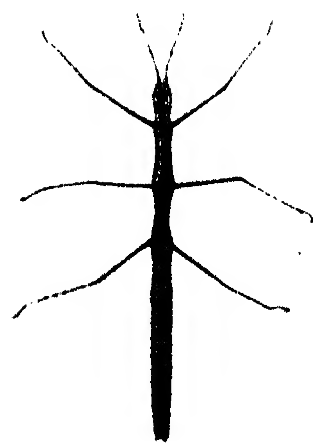


Fig. 5C.



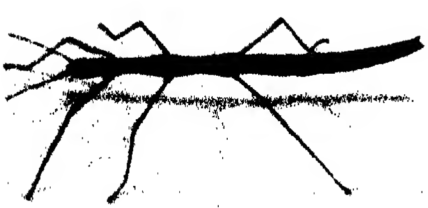


Fig. 5B.

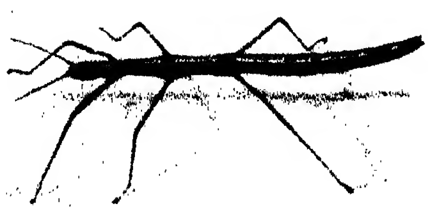


Fig. 5D.

The Rate of Absorption of Various Phenolic Solutions by Seeds of Hordeum vulgare, *and the Factors Governing the Rate of Diffusion of Aqueous Solutions across Semipermeable Membranes.*

By Adrian J. Brown, F.R.S., and Frank Tinker, M.Sc.

(Received August 27, 1915.)

It has been pointed out previously* that the seeds of Hordeum (barley) are enclosed by a membrane which exhibits the exceptional property of differential permeability, and that use may be made of this property of the seeds for the purpose of investigating some of the obscure phenomena associated with osmosis.†

When the dry seeds are immersed in aqueous solutions of most inorganic acids and salts, sugars, etc., water only passes through the containing membrane and moistens their starchy contents; with other classes of solutes, however, such as the phenols, fatty acids, and monohydric alcohols, the solute enters the seeds together with water. A dry seed of Hordeum may be regarded, therefore, as a diffusion system consisting of a mass of solid material capable of absorbing moisture, enclosed within a differentially permeable membrane. In the presence of those solutes which do not enter the seed-diffusion-system along with the solvent the action of its membrane is comparable to that of a copper-ferrocyanide membrane in contact with sugar solution. On the other hand, in the presence of those solutes which do enter the seed-diffusion-system together with the water, its membrane evidences a property of selective permeability only recognised to any extent with living protoplasm, and with the coverings of certain seeds.

Previous investigations carried out by methods of experiment already described have demonstrated that when seeds of Hordeum are immersed in solutions of solutes to which the seeds are impermeable, less water enters the seeds than from pure water, and that the actual amount entering is

* A. J. Brown, "On the Existence of a Semipermeable Membrane enclosing the Seeds of some of the Gramineæ," 'Annals of Botany,' vol. 21, p. 79 (1907); "The Selective Permeability of the Coverings of the Seeds of *Hordeum vulgare*," 'Roy. Soc. Proc.,' B, vol. 81, p. 82 (1909); A. J. Brown and F. P. Worley, "The Influence of Temperature on the Absorption of Water by Seeds of *Hordeum vulgare*,' 'Roy. Soc. Proc.,' B, vol. 85, p. 546 (1912).

† The seed of the sub-species of Hordeum (barley) known as *Hordeum vulgare* has been found especially suitable for experimental purposes, and has been employed in the following investigation.

regulated, in the main, by the osmotic pressure of the solution in which the seeds are immersed—a condition normal to the behaviour of any semi-permeable diffusion system.

On the other hand it has been demonstrated that with solutions of those solutes which are able to diffuse through the seed membrane there is a general tendency not only for more moisture to enter the seeds than from pure water itself, but also for the rate of entry of the moisture to be accelerated to an extent varying with both the nature of the solute and the concentration of its solution.

The factors controlling the rate of entry of moisture into the seeds provide a promising subject for investigation, but so far the influence of temperature only has been studied. It has been shown in a previous communication* that the rate of entry of moisture into the seeds from both pure water and solutions of permeable and impermeable solutes is an exponential function of the temperature with a very high coefficient closely approximating that of vapour pressure, and also of a number of chemical reactions occurring in solution. But this conclusion provides no explanation of the interesting observation that the rate of entry of moisture into the seeds from solutions of permeable solutes varies with the nature of the solute when the solutions are at constant temperature and of equimolecular concentration. Presumably the explanation of this is to be found in the variation in the physical properties of the different solutes when in solution, and it was with the object of discovering, if possible, with which of the physical properties of the solutions the rate of absorption is associated, that the present research was undertaken. Such properties as are likely to exert an influence on the absorption rate are evidently ionisation, osmotic pressure, vapour pressure, viscosity, and surface tension.

Preliminary experiments showed that the phenols would form a most suitable series of solutes for the purpose of the present comparative study. The membrane of the barley seed is permeable to them all; they show striking differences among themselves with regard to some of their physical properties, whilst other of their properties are almost equal, and they have a negligible ionisation when in solution, so that consideration of this latter factor is eliminated when dealing with them. The solutions chosen for the present research accordingly comprised those of phenol, catechol, resorcinol, quinol, and pyrogallol.

Three series of comparative experiments were made with the above phenolic solutions:—

(*a*) In the first series samples of seed were steeped in seminormal

* Brown and Worley, *loc. cit.*

solutions of phenol, catechol, resorcinol, quinol, and pyrogallol at 19° C. Thus the nature only of the steeping solution was different, the temperature and molecular concentration being the same in each case.

(*b*) The second series of solutions was exactly similar to the above, except that the steeping temperature was increased to 32·2° C. This series was chosen in order to determine what difference would be made to the relative rates of absorption by increasing the temperature, whilst keeping all the other factors the same.

(*c*) In the third series the various samples were steeped in solutions of ordinary phenol of various strengths at 22·6° C., in order to determine the effect of altering the concentration of a given solution at a constant temperature.

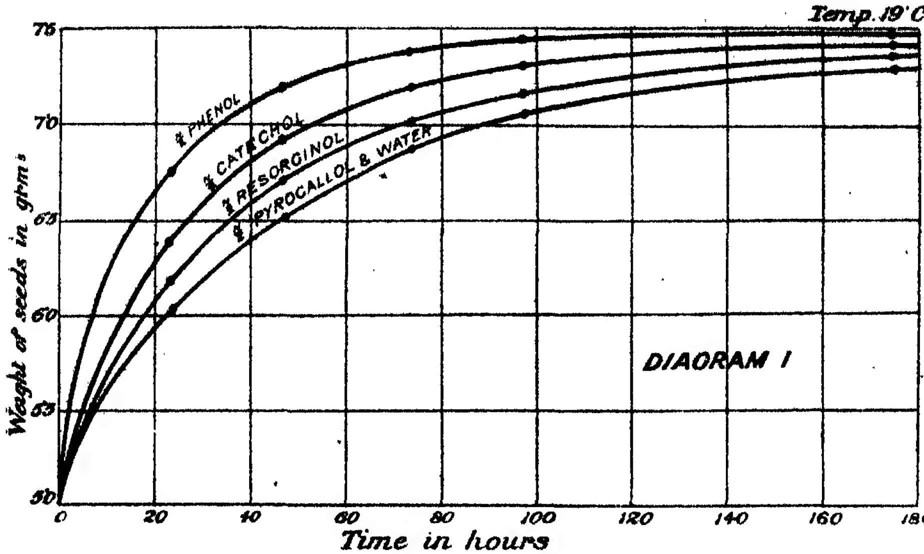
Experimental Determination of the Increase in Weight when the Seeds are Immersed in the Various Solutions.

(a) *Seeds in Seminormal Phenolic Solutions at* 19° *C.*—In this series the solutions were made of equivalent strength (half molar by volume in each case). Since they contained equal numbers of solute molecules their osmotic pressures were consequently equal.* Notwithstanding this fact, however, the rate at which the solution is absorbed varies greatly. This is shown in Table I and its corresponding curve, expressing the weight of the original 5 grm. of seeds after stated intervals.

Table I.—Temperature 19° C.

Time from beginning of experiment.	Weight of seeds immersed in					
	Water.	N/2 phenol.	N/2 catechol.	N/2 resorcinol.	N/2 quinol.	N/2 pyrogallol.
	grm.	grm.	grm.	grm.	grm.	grm.
0 hours	5·00	5·00	5·00	5·00	5·00	5·00
1 "	5·11	5·13	5·12	5·10	5·13	5·16
24 "	6·08	6·73	6·31	6·11	6·15	6·08
48 "	6·56	7·22	6·91	6·66	6·69	6·58
72 "	6·94	7·38	7·24	7·07	7·06	6·96
96 "	7·15	7·44	7·34	7·25	7·29	7·21
7 days	7·31	7·50	7·37	7·39	7·40	7·38

* Assuming, of course, that dilute solutions of equal molecular concentration have equal osmotic pressures. This is only so absolutely when the solutions have also equal heats of dilution and evaporation and equal surface tensions, and when the degree of association of the solvent is the same in each case (Findlay, 'Osmotic Pressure,' Chap. V).



An inspection of the above Table, and its corresponding curve, shows that the rate of absorption gradually falls off in each case as the equilibrium point is reached. But it will be seen also that the time in which equilibrium is attained is considerably shorter for the phenolic solutions than for water. Each of the phenols, with the exception of pyrogallol, exerts a marked accelerating effect on the rate at which its solution is absorbed, the order in which they do this being—(1) phenol, (2) catechol, (3) resorcinol and quinol, (4) pyrogallol and water. The rates of absorption for solutions of resorcinol and quinol, on the one hand, and pyrogallol and water on the other, are practically identical.*

(b) *Seeds in Seminormal Phenolic Solutions at* 32·2° C.—In this series all the essential factors were kept the same as in the previous series, with the exception that the temperature was raised from 19° C. to 32·2° C. A small rise in temperature is accompanied by a great increase in the velocity of moisture absorption. This greatly increased rate is shown in Table II. Whereas the equilibrium is attained only after six or seven days at a temperature of 19° C., it is reached after two or three days at 32·2° C.†

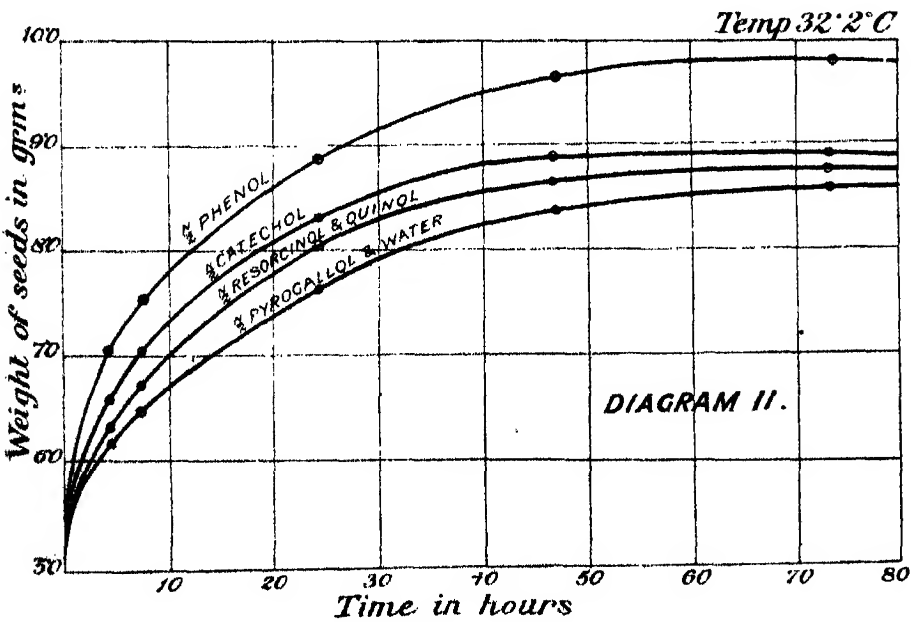
* It is also worthy of note that a high rate of absorption also seems to be accompanied by a slight raising of the equilibrium point; for instance, the weight of phenol absorbed by the seeds when these are in equilibrium with the surrounding solution is 0·2 grm. greater than the weight of water absorbed at equilibrium. This figure is too great to be accounted for by the slightly greater density of seminormal phenol solution.

† The comparison is only a rough one, since the samples of seeds were different ones in the two cases, and the initial moisture-content different also. But it has been shown elsewhere (Brown and Worley, *loc. cit.*) that the velocity of absorption is an exponential function of the temperature, which increases very rapidly.

But, in spite of this general increase in the velocity of absorption, the relative rates at which the solutions are taken up remains the same at 32·2° C. as at the lower temperature. All the phenolic solutions, with the exception of pyrogallol, are absorbed at a greater rate than pure water.

Table II.—Temperature 32·2° C.

Time from beginning of experiment.	Weight of seeds immersed in					
	Water.	N/2 phenol.	N/2 catechol.	N/2 resorcinol.	N/2 quinol.	N/2 pyrogallol.
hours.	grm.	grm.	grm.	grm.	grm.	grm.
0	5·00	5·00	5·00	5·00	5·00	5·00
2	5·99	6·36	6·08	5·99	5·99	5·95
5	6·41	7·03	6·53	6·49	6·49	6·41
8	6·74	7·52	6·94	6·88	6·84	6·74
15	7·26	8·29	7·55	7·45	7·46	7·27
24½	7·77	8·85	8·12	8·04	8·03	7·78
48	8·43	9·30	8·72	8·62	8·65	8·48
72	8·69	9·37	8·86	8·80	8·80	8·71

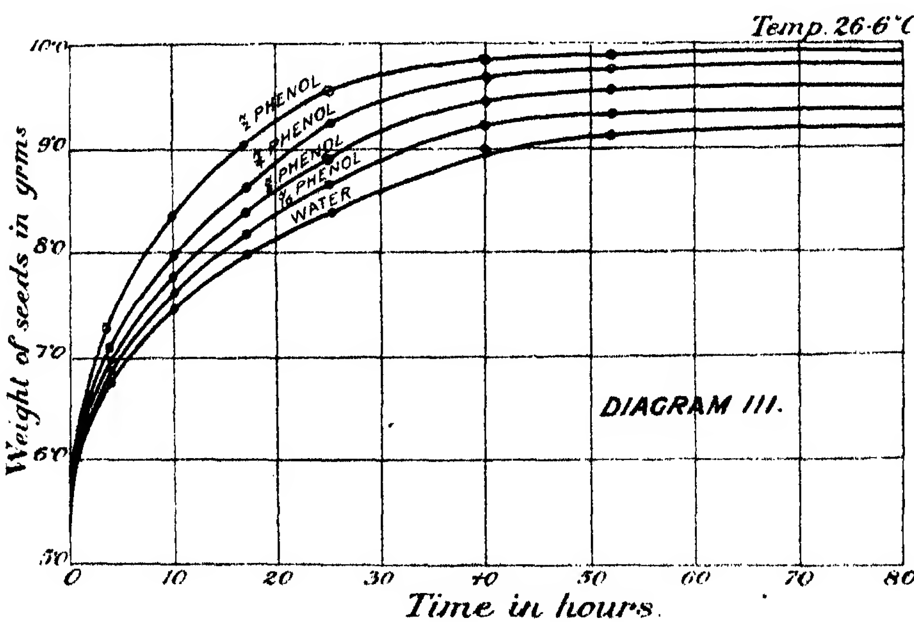


(c) *Seeds in Solutions of Ordinary Phenol of Various Concentrations at* 26·6° C.—As might be expected, the accelerating effect of phenol on the rate of absorption is diminished when the amount dissolved is diminished. This is shown in Table III and the corresponding diagram. The accelerating effect is most marked in the case of seminormal phenol, and the rate of

absorption gradually approaches that of water as the dilution is increased. When the concentration has been reduced to thirty-secondth normal, the velocity of absorption of the solution is practically equal to the rate at which pure water is taken up.

Table III.—Temperature 26·6° C.

Time from beginning of experiment.	Weight of seeds immersed in					
	N/2 phenol.	N/4 phenol.	N/8 phenol.	N/16 phenol.	N/32 phenol.	Water.
hrs.	grm.	grm.	grm.	grm.	grm.	grm.
0	5·00	5·00	5·00	5·00	5·00	5·00
4	7·18	7·03	6·93	6·85	6·78	6·75
10	8·22	7·97	7·80	7·68	7·50	7·46
18	8·95	8·66	8·52	8·34	8·10	8·04
25½	9·35	9·02	8·90	8·72	8·41	8·39
40	9·71	9·38	9·24	9·13	8·96	8·92
51½	9·86	9·47	9·35	9·26	9·19	9·15



The Accelerating Effect of the Phenols on the Rate of Absorption is not due to Disruption of the Membrane.

It might be argued that the remarkably high velocity of absorption of the phenolic solutions by the barley seeds, as compared with water, is due to the

complete or partial disruption of the semipermeable membrane by the solutions. That this is not so was shown in the following way:—

After each sample of seeds had attained fulness in the various seminormal phenolic solutions at 19° C., they were slowly and carefully dried in air at about 30° C. The samples were next immersed in pure water in each case, and the rate of absorption of the pure water determined in the same way as when the seeds were in the phenolic solutions. It is evident that, if any sample had been damaged, it would have absorbed water more quickly than the sample originally in pure water. It was found, however, that the rate of absorption was almost exactly the same in every case as the rate for the sample originally in pure water. A comparison of the corresponding weights, after stated intervals of the two samples originally in water and seminormal phenol, is given in Table IV.

Table IV.—Temperature 19° C.

Time from beginning.	Weight in pure water of seeds originally immersed in		Difference.
	Water.	N/2 phenol.	
days.	grm.	grm.	grm.
0	4·71	4·74	0·03
1	6·10	6·25	0·15
2	6·61	6·71	0·10
3	6·92	6·95	0·03
4	6·97	7·02	0·05
7	6·97	7·10	0·13

It is apparent that the weight differences between the seeds originally in N/2 phenol and water respectively are comparatively small, and within the experimental error involved in prolonged manipulation—losses in weight being caused by numerous dryings with a cloth.

Discussion of the Absorption Curves.

(1) *Equation to the Absorption Curves*—

It has been pointed out already that the rate at which the seeds absorb any solution is dependent on the amount of solution which has previously entered them (their degree of fulness), as well as on the nature of the solution. In order to find the mathematical relationship existing between the absorption rate and the degree of fulness, tangents were drawn to each of the curves in Diagrams 1, 2, and 3 at points where the seeds had attained definite weights

e.g. 6·5, 7·0, 7·5, 8·0 grm.* Table V shows the rate of entry of each of the solutions, in grammes per hour, when the original 5 grm. of seeds have increased in weight to the given extent.

Table V.—Showing the Rate of Absorption (in grammes per hour) of the Various Solutions when the Seeds have attained given Weights.

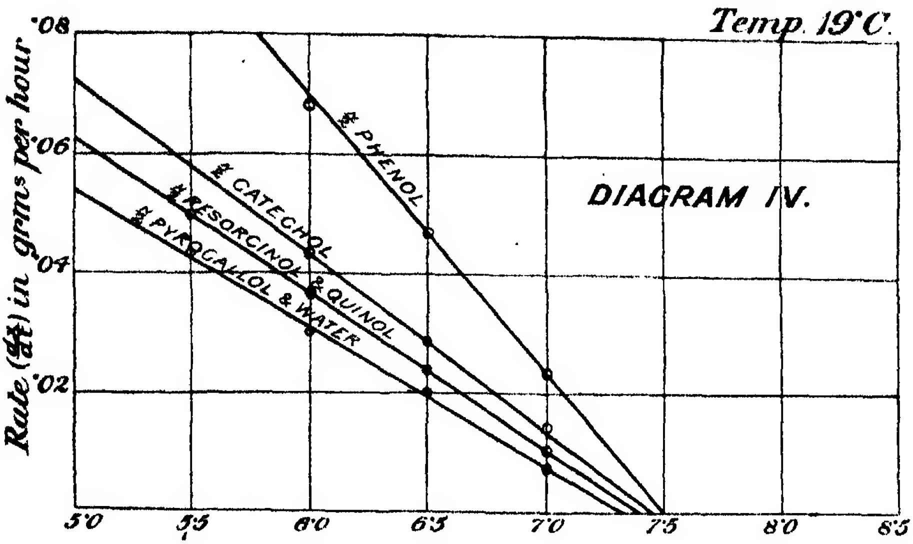
Weight to which original 5 grm. seeds have attained.	Rate of entry (in grammes per hour) of—					
	Pure water.	N/2 phenol.	N/2 catechol.	N/2 resorcinol.	N/2 quinol.	N/2 pyrogallol.
	(a) Seminormal Phenolic Solutions at 19° C.					
grm.						
5·5	0·044	—	—	0·050	0·050	0·046
6·0	0·031	0·068	0·042	0·036	0·035	0·031
6·5	0·020	0·047	0·026	0·022	0·022	0·020
7·0	0·009	0·021	0·015	0·012	0·014	0·011
	(b) Seminormal Phenolic Solutions at 32·2° C.					
6·5	0·100	0·245 ?	0·140	0·131	0·130	0·100
7·0	0·075	0·182	0·105	0·099	0·099	0·075
7·5	0·053	0·147	0·078	0·068	0·068	0·053
8·0	0·032	0·106	0·050	0·039	0·032	0·032
8·5	—	0·070	0·026	0·015	0·015	—

(c) Ordinary Phenol Solutions at 26·6° C.

Weight to which original 5 grm. seeds have attained.	Rate of entry (in grammes per hour) of—				
	N/2 phenol.	N/4 phenol.	N/8 phenol.	N/16 phenol.	Water.
grm.					
7·0	0·245	0·205	0·170	0·155	0·120
7·5	0·195	0·160	0·135	0·115	0·090
8·0	0·150	0·115	0·105	0·085	0·060
8·5	0·105	0·075	0·070	0·055	0·035
9·0	0·065	0·035	0·030	0·025	0·010

Diagram 4 shows graphically the results for the seminormal phenolic solutions at 19° C. The diagrams for the other solutions are exactly similar in form.

* The rate of entry of the solution also falls off as the time proceeds, but this is obviously because they are all the while getting fuller. It is evident that the time is only a secondary factor ; the degree of fulness, as indicated by the weight to which the seeds have attained, being the fundamental one.



In the case of every solution studied, there is evidently a straight line relationship existing between the absorption rate and the degree of fulness. The rate of entry at any given time is therefore given by an equation of the type

$$dx/dt = k(a-x),$$

where

dx/dt = rate of increase in weight (in grammes per hour) when the seeds have attained the weight x;

x = weight of the seeds after the interval t;

a = weight of the seeds at equilibrium;

k = a constant for the solution at the given temperature and concentration.

The values of the constants k and a are given in Table VI.

Table VI.

	Temp. 19° C		Temp. 32·2° C.	
	k.	a.	k.	a.
(a) Seminormal Phenolic Solutions.				
		grm.		grm.
Water	0·024	7·34	0·045	8·7
N/2 phenol	0·047	7·50	0·076	9·4
N/2 catechol	0·029	7·42	0·056	8·9
N/2 resorcinol	0·026	7·37	0·052	8·8
N/2 quinol	0·026	7·37	0·052	8·8
N/2 pyrogallol	0·024	7·34	0·045	8·7

Table VI—*continued.*

(*b*) Ordinary Phenol Solutions of Various Strengths at 26·6° C.		
	k.	a.
		grm.
N/2 phenol	0·090	9·8
N/4 "	0·082	9·5
N/8 "	0·073	9·4
N/16 "	0·064	9·3
Water	0·054	9·2

Since the relationship between the absorption rate and the degree of fulness is a linear one, it follows that the original experimental absorption curves are logarithmic.

Integration of the equation

$$dx/dt = k(a-x),$$

between the limits x and x_1, gives

$$\log_e \frac{a-x_1}{a-x} = k(t-t_1).$$

Taking x_1 as the initial weight (5 grm.), this becomes (since $t = 0$)

$$\log_e \frac{a-5}{a-x} = kt.$$

This equation represents the experimental absorption curves with a high degree of accuracy. If the weight of the original 5 grm. of seeds after any given interval is calculated from it, there is rarely a difference exceeding two or three tenths of a gramme between the calculated and the actual experimental value. Table VII gives this comparison for the seeds immersed in water at 19° C., and also those steeped in N/2 phenol at the same temperature. The differences between the calculated and the experimental values are well within the experimental errors involved in weighing and drying the seeds.

The absorption equation for water at 19° C. is

$$\log_e \frac{2{\cdot}34}{7{\cdot}34-x} = 0{\cdot}024\,t,$$

and for N/2 phenol

$$\log_e \frac{2{\cdot}5}{7{\cdot}5-x} = 0{\cdot}045\,t.$$

Table VII.

Time from beginning of experiment.	Seeds in water at 19° C.			Seeds in N/2 phenol at 19° C.		
	Calculated.	Experimental.	Difference.	Calculated.	Experimental.	Difference.
hours.	grm.	grm.		grm.	grm.	
0	5·00	5·00	—	5·00	5·00	—
24	6·03	6·08	+0·05	6·64	6·73	+0·09
48	6·59	6·56	−0·03	7·20	7·22	+0·02
72	6·93	6·94	+0·01	7·40	7·38	−0·02
96	7·11	7·14	+0·03	7·46	7·44	−0·02

(2) *The Absorption Constant k, and the Interpretation of the Absorption Equation.*

The equation $dx/dt = k(a - x)$ is worthy of consideration in more detail. It shows that the rate of absorption of a solution by the barley at any given instant is the product of two factors—the constant k, which depends on the nature of the solution, its temperature and concentration; and the degree of dryness $(a - x)$ of the seeds. These two separate factors are considered more fully below.

(a) *Effect of the Degree of Hydration of the Internal Contents of the Seeds on the Absorption Rate.*—The term $a - x$ represents the difference between the equilibrium weight and the weight x at any given instant. It follows therefore, from the above equation, that when the seeds are surrounded by a solution of constant concentration and temperature, the rate of absorption of the solution by the barley is proportional to the weight of the solution which the seeds are still capable of absorbing before equilibrium is reached. For convenience this weight might be termed the degree of dryness of the seeds. The rate of absorption is a maximum when the seeds contain no moisture at all, and zero when the seeds are full.

The gradual falling off of the rate of absorption as the degree of fulness gets greater seems to be caused by the operation of a backward pressure;* for it is evident that the solution would of itself tend to diffuse into the seeds at a constant rate, since its temperature and concentration remain the same throughout. But as it flows into the seeds, the gradually increasing vapour pressure of the hydrated starchy contents of the seeds opposes to a

* The existence of this backward diffusion pressure is amply proved by the fact that wet seeds gradually give up their moisture in dry air, or when immersed in anhydrous alcohol, sulphuric acid, or other media to which the membrane is impermeable. The rate of diffusion of the moisture outwards gets less as the seeds get drier, and finally becomes zero.

progressively greater extent the forward diffusion pressure of the solution outside. The rate of absorption of the solution is at a maximum when the seeds are absolutely dry, because the backward pressure tending to make the solution diffuse out again is then zero. It is zero at equilibrium because the backward diffusion pressure has then become equal to the forward diffusion pressure of the solution outside.

(b) *Effect of the Nature of the Solution, etc., on the Absorption Rate: the Absorption Constant.*—It has been indicated already that, in order to compare the relative rates at which various solutions are absorbed by the seeds, equal degrees of fulness x should be chosen. But if x is given a constant value for all the solutions in any series, the value $a-x$ for all the solutions will be the same also, since the magnitude of the equilibrium weight a is practically the same in every case (see Table VII). It would follow, therefore, that the relative rates of absorption of the various solutions at any given degree of fulness (x) are proportional to their constants k. The constant k might hence be termed the absorption constant for the given solution. It gives a measure of the relative rate at which the solution will enter the seeds at all degrees of fulness. Thus, whatever weight the seeds may have attained, seminormal phenol always enters the seeds with about twice the velocity that water enters, since its absorption constant is about twice that of water.

An inspection of Table VI shows that the absorption constants of all the phenolic solutions are greater than that of water, with the exception of pyrogallol, which has a constant practically the same as that of water. The constant increases greatly in value with a slight rise in temperature. This increase is an exponential one rather than linear, being like vapour pressure in this respect. Indeed it has been shown* that the curves showing the relationship between the rate of entry of water into the seeds and the temperature can almost be superposed on the vapour pressure–temperature curves for water, either being represented by the equation

$$v = ce^{\kappa\theta},$$

where v represents either the velocity of absorption of water or the vapour pressure, and θ the temperature, k and c being constants.

Since it has now been shown that the rate of absorption is always proportional to the absorption constant, it follows that the latter is an exponential function of the temperature, *i.e.*

$$k \propto ce^{\kappa\theta},$$

where κ has the same value as in the temperature–vapour pressure curves. In other words, if a series of temperatures be chosen, the absorption

* A. J. Brown and F. P. Worley (*loc. cit.*).

constants of any solution at those temperatures are approximately proportional to the corresponding vapour pressures. This result would seem to suggest that there is intimate relationship between the absorption constant and the vapour pressure of a solution. It will be shown later that there is also a close connection between the absorption constant and the surface tension. Apparently the absorption constant is one of the most important constants for a solution in contact with a selectively permeable membrane.

Relation between the Rate of Diffusion of the various Phenolic Solutions across the Barley Membrane and the Physical Properties of the Solutions.

It appeared to be of interest to correlate the rate of absorption with the common physical properties which might exert an influence on the rate of diffusion of a solution across the barley membrane. Such properties were evidently osmotic pressure, vapour pressure, viscosity and surface tension. Accordingly these properties were measured for the solutions in question, with the exception of osmotic pressure. The latter was taken as being practically identical for the seminormal phenolic solutions.* Any slight differences there might be in them were obviously unable to account for the great variations noticed in the magnitude of the absorption constants. Further, if the osmotic pressure of the solution were the predominating factor in determining the rate of diffusion across the membrane, the osmotic pressures of the phenolic solutions ought to be negative, since they diffuse across the membrane at a greater rate than pure water. The existence of a solution with a negative osmotic pressure is most unlikely.†

(a) *Surface Tensions of the Solutions.*—The surface tensions of the solutions were measured by the capillary tube method, using a similar apparatus to that employed by R. P. Worley in the determination of the surface tensions of aqueous solutions of aniline and phenol.‡ The correct temperature was attained by immersing the solutions in a thermostat with glass sides for a few hours. The capillary rise was measured by means of a vertical cathetometer reading to 1/10 mm., whilst the capillary diameter was measured on the microscope stage by means of a graduated eyepiece. Instead of determining the average diameter of the capillary it was thought advisable to determine this at the point where the meniscus had been, a procedure which

* See also footnote on p. 121.

† The equality of the partial vapour pressure of water in solutions of ordinary phenol to the vapour pressure of pure water (Table XI) would indicate that the osmotic pressures of phenol solutions are zero.

‡ R. P. Worley, 'Chem. Soc. Journ.,' vol. 105, p. 260 (1914).

was very simply done by cutting the capillary tube at that point. The formula employed was

$$s = \frac{ghrd}{2},$$

where $g = 981$ cm./sec.^{2}, h = capillary rise in cm., r = capillary radius in cm., d = density of solution, s = surface tension of the solution in dynes/cm.

For the sake of brevity the results only are given. They are correct to the nearest one or two units only, since the error in the determination of the capillary rise and the capillary radius might have amounted to 1 per cent. in each case. But a greater accuracy than this was not necessary for the present purpose.

Table VIII.—Showing Surface Tensions of the Phenolic Solutions.

	Surface tension.			Surface tension at 26·6° C.
	Temp. 19° C.	Temp. 32·2° C.		
	dynes/cm.			
Water	71·8	70·6	N/2 phenol	43·8
N/2 phenol	43·8	41·7	N/4 ,,	49·5
N/2 catechol	56·8	56·3	N/8 ,,	55·2
N/2 resorcinol	63·8	60·0	N/16 ,,	64·8
N/2 quinol	63·7	59·3	N/32 ,,	69·6
N/2 pyrogallol	69·8	69·2	Water	71·5

If the surface tensions in the above Table are compared with the corresponding absorption constants given in Table VI it will be seen that there is a remarkable relationship between the two. The order in magnitude of the absorption constants is the same as the inverse order of the surface tensions in the case of all three series. When the surface tension is abnormally low the absorption constant is abnormally high. Seminormal phenol solution, for example, has a surface tension about half that of water, and an absorption constant about twice as large. Indeed, the absorption constant is approximately inversely proportional to the surface tension of the solution, and this holds whatever the temperature may be. The product of the surface tension and the absorption constant is therefore practically constant. This is shown for the seminormal phenolic solutions at 19° C. in Table IX.

Table IX.

	Absorption constant k at 19° C.	Surface tension at 19° C.	Product constants at 19° C. $k \times$ surface tension.
Water	0·024	71·8	1·72
N/2 phenol	0·045	43·3	1·94
N/2 catechol	0·029	56·3	1·64
N/2 resorcinol	0·026	63·8	1·66
N/2 quinol	0·026	63·7	1·65
N/2 pyrogallol	0·024	69·8	1·68

In the same way the product constant for the seminormal phenolic solutions at 32·2° C. has an approximate value of 3·1, and for the solutions of ordinary phenol of various strengths a value of about 4·0.*

(b) *Viscosity.*—The viscosity of the solutions was measured by means of an Ostwald viscometer of about 20 c.c. capacity, the correct temperature being maintained by immersing it in the same thermostat as was used for the surface tension determinations. The relative viscosity only was found, in terms of water at the same temperature, the usual formula

$$n = t'd'/td$$

being employed; t' and d' being the time of flow and density for the solution; t and d representing the same for water.

The results are given in Table X. An inspection of them shows that the variation in viscosity of the solutions between themselves is too slight to account for the large variations in the absorption rates. Apparently, the viscosity of the solution is a factor of no importance; and even if it exerts a small effect on the rate of diffusion across the membrane, it is completely masked by other factors. Indeed, all the phenolic solutions, which have a slightly greater viscosity than water, tend to diffuse across the membrane more rapidly than the latter.

Table X.

	Viscosity.			Viscosity at 26·6° C.
	Temp. 19° C.	Temp. 32·2° C.		
Water	1·000	1·000	N/2 phenol	1·110
N/2 phenol	1·111	1·099	N/4 „	1·066
N/2 catechol	1·122	1·119	N/8 „	1·051
N/2 resorcinol	1·119	1·118	N/16 „	1·012
N/2 quinol	1·120	1·117	N/42 „	1·001
N/2 pyrogallol	1·129	1·130	Water	1·000

* Since the samples of seeds were different and the initial moisture-content not the same, these constants are not comparable with one another.

(c) *Vapour Pressure.*—In view of the predominance which has been given to vapour pressure in various theories of the mechanism of osmosis, it seemed of interest to obtain data with respect to the vapour pressures of aqueous phenolic solutions. Owing to the experimental difficulties, and the somewhat complicated apparatus required, it was not possible to measure these in the present research. The nearest results available are those given by Schreinemakers,* which refer to aqueous solutions of phenol at 56·3° C. This temperature is considerably higher than those worked with in the case of barley, and on this account the results are not absolutely comparable. The surface tensions of the solutions are given side by side, for purposes of comparison.†

Table XI.—Vapour Pressures and Surface Tensions of Solutions of Phenol at 56·3° C.

Percentage of phenol.	Total vapour pressure.	Partial vapour pressure of water.	Surface tension.
	mm.	mm.	
0·0	125	125	66·7
2·0	125	125	52·5
5·58	127	125·5	42·0
7·42	126·5	125	39·4

Since the law that the absorption constant is inversely proportional to the surface tension seems to hold for all temperatures,‡ it would follow that, even at 56·3° C., the absorption constant for phenol would be large relative to water, since the surface tensions are still widely different at that temperature (see above Table). On the other hand, both the total and partial vapour pressures of the phenol solutions of all concentrations differ very slightly from the vapour pressure of pure water, and it will be seen that, although the total vapour pressures of the phenol solutions are very slightly greater than the vapour pressure of pure water, they are by no means great enough to account for the large differences in the rates of absorption.

Conclusions.

It is apparent from what has preceded that the variations in the rate at which phenolic solutions diffuse across the differentially permeable membrane of barley are primarily associated with differences in the surface tensions of the solutions. In the case of the phenolic solutions studied, the surface tension is the only physical property whose variation is great enough to

* Schreinemakers, 'Roy. Acad. Amsterdam Proc.,' vol. 3, pp. 1 and 701.

† The surface tensions are taken from R. P. Worley's paper (*loc. cit.*).

‡ See p. 132.

account for the relative differences in the absorption constants. At any given temperature the magnitudes of the other physical properties of the aqueous phenolic solutions are practically the same. This does not mean that these factors have no influence at all on the rate of absorption. Indeed, they must exert a more or less pronounced influence on the rate of diffusion of a solution across a semipermeable membrane. Bartrell, for instance, has shown* that solutions diffusing through copper ferrocyanide and other membranes obey Poiseuille's law, according to which the rate of diffusion of a solution across a membrane would be inversely proportional to its viscosity. It has also been shown in the case of barley that if a series of temperatures are chosen, the rate of diffusion of moisture into the seeds is proportional to the corresponding vapour pressures.

The only legitimate conclusion to draw from the preceding work is that when the osmotic pressures, vapour pressures, and viscosities of a series of solutions of *permeable* solutes are equal, their rates of diffusion across the barley membrane are inversely proportional to their surface tensions.

A close relationship between the rate of diffusion of solutions of permeable solutes and their surface tensions might be anticipated. When a solution has a lower surface tension than the pure solvent, it has also a lower intrinsic pressure. In terms of the Laplace theory of capillarity this implies that, by some means or another, the solute molecules have diminished the attractive power of the solvent molecules for one another. The solvent molecules will therefore tend to diffuse over the surface of the barley membrane all the more readily, and from thence on to the surfaces of the solid particles of absorbent material within the seeds.†

These considerations must be confined, however, to solutions of solutes which are permeable to the membrane, and not extended to osmotic phenomena as a whole, as I. Traube has attempted to do.‡ Driving forces due to differences in osmotic pressure on the two opposite sides of the membrane must be eliminated. This is not the case when the membrane is impermeable to the solute and pure water alone enters the seed.

* Bartell, 'J. Phys. Chem.,' vol. 15, p. 659 (1911).

† The relationship between the intrinsic pressure, or "attraction pressure," and other physical properties such as surface tension, lowering of vapour pressure, compressibility of solution, etc., has been worked out in detail by I. Traube ('J. Phys. Chem.,' vol. 14, p. 452 (1910), and many other papers). In particular low surface tension, and therefore low intrinsic pressure, is accompanied by abnormally high vapour pressure. For instance, it has been shown in Table XI on p. 134 of the present paper that the partial vapour pressure of water in phenol solutions is equal to that of pure water. The fact that it is not *greater*, however, shows incidentally that the moisture does not pass across the membrane as vapour.

‡ I. Traube, *loc. cit.*

The Controlling Influence of Carbon Dioxide. Part III.—*The Retarding Effect of Carbon Dioxide on Respiration.*

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Introduction.

In Parts I and II of these researches* we have been led to the conclusion that the resting condition of the moist seed, so often occurring in nature, is primarily a phase of autonarcosis under the action of the carbon dioxide produced by the seed itself. It has been shown that retardation and suspension of normal activity in plant protoplasm is produced by carbon dioxide in conditions otherwise entirely favourable to growth and during a stage normally characterised by vigorous growth. Attention has already been called to the striking analogy existing between the dormancy of the non-growing moist seed under the influence of carbon dioxide, whether maturing on the parent plant or showing delayed germination in the soil, and the dormancy of the unfertilised ovum. In both cases apparently simple causes are found sufficient to produce a change in the cell conditions owing to which the cell or tissue passes from dormancy into active growth by cell division. In neither case are the factors conditioning dormancy on the one hand and growth by cell division on the other as yet clearly established. A knowledge of these factors, however, must be of great

* "The Controlling Influence of Carbon Dioxide in the Maturation, Dormancy and Germination of Seeds.—Parts I and II," 'Roy. Soc. Proc.,' B, vol. 87, pp. 408 and 609.

importance both in physiology and medicine. In studying the phenomenon of carbon dioxide inhibition, as exhibited by dormant seeds, we seem to be in the presence of that fundamental question in physiology, the question of the cause of growth.

Our problem, then, in these researches is clearly to determine what physiological changes accompany the inhibition of growth under the influence of carbon dioxide. Two hypotheses may be put forward. The inhibitory action of carbon dioxide may be the indirect result of a carbon dioxide effect upon the physical state of the protoplasm, for example, a change in its colloidal structure, its water-holding capacity, its permeability. On the other hand it may be of the nature of a direct chemical action producing a change in some phase of metabolism. Work to determine how far effects of the first class actually exist is still in progress. In the present paper one striking effect of the second class will be dealt with, namely, the retarding effect of carbon dioxide upon respiration. That oxidation and respiration are necessary for growth by cell division in plants is known. With regard to the animal ovum Loeb finds that oxidations are a controlling factor in the stimulus to growth, whether by natural or artificial fertilisation. The breaking up of the dormant condition of the egg and the beginning of cell division is accompanied by an essential increase in respiration. Should this increase be prevented either by a small dose of potassium cyanide or by the absence of oxygen, fertilisation, whether artificial or natural, has no effect, and growth does not ensue.

It is a simple assumption at the outset that the presence of carbon dioxide may interfere with the forward movement of the respiratory process producing CO_2. From much recent work it is clear, however, that respiration is by no means a simple process, but that it includes a complicated chain of reactions. A short reference therefore of some results already obtained in work upon plant respiration and of the conclusions based upon them will be useful before proceeding to consider the results described in the following pages.

Section I.—*The Complexity of the Respiratory Processes in Plants.*

The discovery, first made by Rollo in 1798 but not widely accepted for many years, that plant tissues will produce CO_2 in the absence of oxygen as a vital process, opened up a promising line of enquiry under the influence of a leading idea, namely, that this anaërobic CO_2 production was intimately associated with processes of normal respiration. Pfeffer believed that this anaërobic CO_2 production, which he termed intramolecular respiration, was a normal process in plant respiration, being genetically connected with sequent

oxidations by the oxygen of the atmosphere under ordinary conditions. Intramolecular respiration was thought to be essentially the same as alcoholic fermentation. There appeared, however, objections to Pfeffer's theory, notably that the amount of CO_2 produced in anaërobic respiration was found not to bear a definite relation to the amount of CO_2 produced in normal respiration, that is to say a relation of one to three, as should be expected from Pfeffer's view. Further, plant tissues did not appear to have the power of oxidising alcohol.

In 1903 this line of speculation received a renewed stimulus. Stoklasa and Czerny reported the extraction of an enzyme from beetroots, potatoes, peas and other plant tissues, and also from animal tissues such as the liver, heart, lungs and kidney, which they termed zymase and which appeared quite similar to Buchner's enzyme, extracted from yeast a few years previously. Following these discoveries, attention was again turned to the study of intra-molecular respiration, but in the result the proportion of carbon dioxide to alcohol formed in anaërobic conditions could not be found to fulfil the requirements of the theory which compared the processes of anaërobic carbon dioxide production strictly to those of fermentation in the presence of zymase. The amount of CO_2 produced generally exceeded the amount theoretically required in relation to the alcohol formed. It was concluded that the CO_2 evolved in anaërobic respiration arose in varying degrees from other sources as well as from the action of zymase.

At the present day, with the researches of Blackman, Palladin, Deleano, and others, we have advanced to a more critical view of the various processes underlying plant respiration. It has been recognised that under the general heading of respiration we are dealing with the combined result of various distinct processes. A leading distinction has been pointed out by Blackman between that respiration on the one hand which involves the breakdown of sugar, fat, or organic acid, which he has termed floating respiration, and on the other hand the essential respiration of protoplasm. The present position of research with regard to the mechanism of plant respiration appears to be that we find ourselves faced with a complexity in time, on the one hand, in which a chain of sequent reactions is involved and on the other hand with a complexity in space in which various types of material are being simultaneously broken down along different paths. The present researches confirm this conception.

Section II.—*Methods of Experiment.*

In these experiments, which have been directed to determine the influence of carbon dioxide upon respiration, the general method adopted was as follows.

Experiments were conducted in parallel series, each parallel series consisting of a number of experiments set up at the same time with varying percentages of CO_2 present and a control. The respiring material was enclosed in pressure flasks of about 1000 c.c. capacity closed by rubber corks or by specially constructed ground glass stoppers and a mercury seal. The desired atmospheres were made up, after evacuating the flasks with material in place, from cylinders of compressed gas, measured in by means of a manometer. Nitrogen was used as the residual gas in order to conduct all the experiments at atmospheric pressure. In the case of seeds a little wet sterilised sand was put at the bottom of the flasks, the amount of sand and water being carefully equalised for experiments of the same series, and the dry seeds were set on this. Before the first gas estimations were made a short interval of time was allowed for a physical equilibrium to be reached between the water in the flasks and the partial pressures of CO_2 introduced. In the case of leaves a longer period was allowed before the first analysis or pressure reading.

A difficulty encountered in the experiments was to obtain accurate measurements of small increases of CO_2 relatively to large partial pressures of the same gas initially present. Two different methods were employed in estimating the CO_2 produced. It was calculated from analyses of small samples withdrawn at intervals, or, in the case of the anaërobic experiments, it was calculated from the increased pressures created in the flask. Manometers are necessary in either case, since to obtain comparable results from analyses the pressures of the gas mixtures must also be known. Open mercury gauges were used and corrections made for the barometer variation. The highest pressure changes recorded were not more than $\frac{1}{10}$ of an atmosphere, *i.e.*, 76 mm.

The gas analyses were made with Haldane's apparatus, 10 c.c. samples being withdrawn, that is from 1 to 2 per cent. of the total gas present. The withdrawal of these samples, though it does not alter the proportions of the various gases present, decreases their active mass or pressure. With regard to carbon dioxide this decrease is always more than compensated by the CO_2 evolved in respiration. Only where large percentages of CO_2 are present does the amount lost in any analysis come near to equalising the amount produced by seeds or leaves in the interval since the previous analysis. Generally speaking, then, the pressures of CO_2, the effect of which upon respiration we wish to find, rise continually while an experiment lasts. This rise is determined by the same readings which give us the amount of CO_2 produced in respiration, so that the data obtained are sufficient for us to calculate figures for the effect of any constant pressure of carbon dioxide.

It is, of course, the value of the active mass or absolute pressure of carbon

dioxide present which must be considered as the effective value in producing the observed retardation of respiration in any case. In our experiments, however, in view of the fact that the changes in total pressure are relatively small, we have taken the percentage figure for carbon dioxide as expressing its effective value. A correction for this error would slightly steepen our curves.

It must be further pointed out that it is the tissue-pressure of CO_2 which is ultimately effective. The tissue-pressure will be higher than the external pressure of CO_2, according to the rate of CO_2 production in the tissues. The data obtained are sufficient to correct for this. As it seemed doubtful whether sufficient accuracy in observation had been obtained to make this correction worth while, it has not been made. The result of such a correction would be again to slightly steepen our curves.

The results are given throughout in cubic centimetres of gas at 0° C. and 760 mm. pressure of mercury.

Section III.—*The Influence of Carbon Dioxide upon Anaërobic Respiration in Seeds.*

In the following experiments living seeds in germinating conditions and also maturing seeds were used as material, the effect upon anaërobic respiration (that is to say, CO_2 production in tissues in the absence of oxygen) of increased concentrations of CO_2 in an atmosphere was determined.

Table I.—Showing the Retarding Influence of Increased Concentrations of Carbon Dioxide upon the Rate of Anaërobic Respiration in Peas (without Testas), otherwise in Germinating Conditions save for the Absence of Oxygen.

Initial CO_2 in atmosphere of N_2 and CO_2	Total anaërobic CO_2 production.				Final CO_2 in atmospheres.
	After 22 hours.	After 50 hours.	After 72 hours.	After 146 hours.	
per cent.	c.c.	c.c.	c.c.	c.c.	per cent.
0	16	33	58	171	·14
28	9	27	41	111	35
48	3	8	13	45	50

Conducted in dim diffuse light. Early Sunrise peas, without testas, set dry on wet sand, 45 c.c. sand, 21 c.c. tap water, 10 peas in each experiment.

CO_2 production estimated from analysis.

Temperature, 20·5° C. thermostat.

In the foregoing Table (I) of results it will be at once seen that increased concentrations of CO_2 have a marked effect in decreasing the anaërobic

production of CO_2 by peas otherwise in germinating conditions. The results of two further similar series of parallel experiments are given in the following Tables II and III. In one of these series green maturing peas just

Table II.—Showing the Retarding Influence of Increased Concentrations of Carbon Dioxide upon the Rate of Anaërobic Respiration in Peas (with Testas), otherwise in Germinating Conditions save for the Absence of Oxygen.

Initial CO_2 in atmospheres of N_2 and CO_2.	Total anaërobic CO_2 production calculated from increased pressures.					
	After 17 hours.	After 23 hours.	After 41 hours.	After 50 hours.	After 65 hours.	After 89 hours.
per cent.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
0	10·3	19·8	61·3	68·8	87·8	108
10	7·5	11·5	37·5	51·5	76	92
20	9	12	35·1	42·5	56·5	72
40	5·2	8	32·7	40	53·4	63·5
60	3·7	6·8	30·8	33	43·5	49·6
80	2·5	3·7	30·7	31·5	42·2	53·1

Conducted in dim diffuse light. In each experiment 25 Early Sunrise peas, with testas, weight 8 grm., set dry in 12 c.c. distilled water, being just sufficient to allow them to fully swell. Peas and flasks sterilised with bromine.

CO_2 production estimated from increased pressure by manometer.

Three hours allowed before first reading.

Temperature, 20° C. thermostat.

Table III.—Showing the Retarding Influence of Increased Concentrations of Carbon Dioxide upon the rate of Anaërobic Respiration in Green Peas (*i.e.*, Peas taken before drying from Living Pods).

Initial CO_2 in atmospheres of N and CO_2.	Total anaërobic CO_2 production.			Final CO_2 in atmospheres after 65 hours.
	After 27 hours.	After 65 hours.		
	From pressures.	From analyses.	From pressures.	
per cent.	c.c.	c.c.	c.c.	per cent.
1	55	103	99	9·6
10·75	48	107	101	18·65
26·6	46	88	85	32·45
79·8	36	—	63	83·9

Conducted in dim diffuse light. In each experiment, 25 fully swelled green peas removed from testa 24 hours before experiment, and 2 c.c. tap water.

CO_2 production calculated (1) from analyses, (2) from increased pressures in flasks. A very fair agreement will be observed in the figures in the Table obtained by the two methods of estimating CO_2 produced.

Temperature 25·5 C., by thermostat.

taken from the parent plant were used as material, in the other dried peas, set in germinating conditions save for the absence of oxygen. In both these cases it is seen that the anaërobic CO_2 production has been reduced in relation to the amount of carbon dioxide present in the atmospheres. Quite similar results were also obtained with white mustard seeds.

In the foregoing experiments, the first fact which stands clearly out is that the anaërobic output of CO_2 varies in some inverse relation to the amount of CO_2 present in an atmosphere. It would clearly be possible to attribute these graded productions of CO_2 to different degrees of disorganisation of the CO_2 producing mechanism. If any such permanent disorganisation is produced its effect should become visible in lowered respiration when the seeds are removed from the excess of CO_2 back to pure nitrogen. This question was tested experimentally. Estimations of CO_2 production were continued after the material had been returned to nitrogen or air free from CO_2. It was found as the result that the CO_2 production, in any parallel series of experiments, returned to an equality in all cases with that of the control in which no CO_2 had been present from the beginning (Tables IV and V).

Table IV.—Showing that Normal Respiratory Activity is Resumed after Removal of Retarding Concentrations of Carbon Dioxide.

Initial CO_2 in N_2 and CO_2.	Anaërobic CO_2 production.								
	In the presence of CO_2 concentrations.								After removal of CO_2 concentrations.
	Estimated from increased pressure after							Checked by analyses after 90 hrs.	Estimated by analyses after further 47 hrs.
	19 hrs.	24 hrs.	31 hrs.	44 hrs.	51 hrs.	67 hrs.	90 hrs.		
per cent.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
0	27	33	39	47	60	66	83	89	41
10	21	25	30	37	48	52	69	74	44
20	19	21	26	32	42	45	60	62	43
40	14	16	20	24	33	35	48	46	43
80	9	10	13	16	24	26	39	34	50

Conducted in dim diffuse light. In each experiment 25 Early Sunrise peas, dry weight 8 grm. set dry in 12 c.c. boiled distilled H_2O, being just sufficient to allow them to fully swell. Peas and flasks sterilised with bromine. Two hours allowed before first reading. Temperature 20° first 20 hours, after that laboratory temperature 16–18° C.

Table V.—Showing that Normal Respiratory Activity is Resumed after Removal of Retarding Concentrations of Carbon Dioxide.

Initial CO_2 in N_2.	CO_2 production. (Anaërobically) in atmospheres of N_2 containing various partial pressures of CO_2.			(Anaërobically) in nitrogen, replacing former atmospheres in each experiment.	(Aërobically) in air replacing former atmospheres in each experiment.	Seeds used germinating finally in air.
	21 hrs.	36 hrs.	45 hrs.	21 hrs.	43 hrs.	Germination, per cent.
per cent.	c.c.	c.c.	c.c.	c.c.	c.c.	
0	33	49	68	26	97	92
10	29	39	51	31	103	96
20	22	32	46	27	99	76
50	18	29	38	24	90	92

Conducted in dim diffuse light. In each experiment, 25 peas with testas, 8 grm. dry weight, set dry with 12 c.c. distilled water.

Anaërobic CO_2 production estimated from pressure increases, aërobic from analyses.

Temperature of experiments 20° C. thermostat.

Peas sterilised in 1/500 bromine for half an hour. Flasks were also sterilised with bromine solution.

These experiments make it clear that the results so far obtained cannot be regarded as due to a permanent injury produced by the increased concentrations of carbon dioxide used. Thus it is seen in Table IV that, while the amounts of carbon dioxide produced by the respiring seeds under the influence of rising percentages of CO_2 form a falling series in some inverse relation to these rising concentrations, yet when subsequently the increased percentages of CO_2 are removed and the conditions in all the experiments equalised, the amount of CO_2 respired by the seeds in each then returns to an equality, as the figures in the sixth column of the Table will show. The conclusion is reached, therefore, that the effect of increased concentrations of CO_2 in the atmosphere is to reduce the CO_2 production in aërobic conditions without destroying the potentiality of the process returning to the normal on the removal of the retarding CO_2 pressures.

This conclusion being established in its general aspect, it is now of interest to examine our results more in detail and quantitatively. In fig. 1 the results of the series of experiments given in Table IV are shown graphically, so as to bring out the depressant action of CO_2. The curves 1–4 represent total CO_2 evolved in cubic centimetres after 19, 31, 51, 90 hours respectively as influenced by the concentration of CO_2 present. Each vertical series of points comes from one experiment. In plotting these,

the mean concentration of CO_2 between the initial and final concentrations for any period between two analyses is taken as the effective pressure influencing CO_2 production during that period. This figure enables one to read values for CO_2 production corresponding to any concentrations of CO_2.

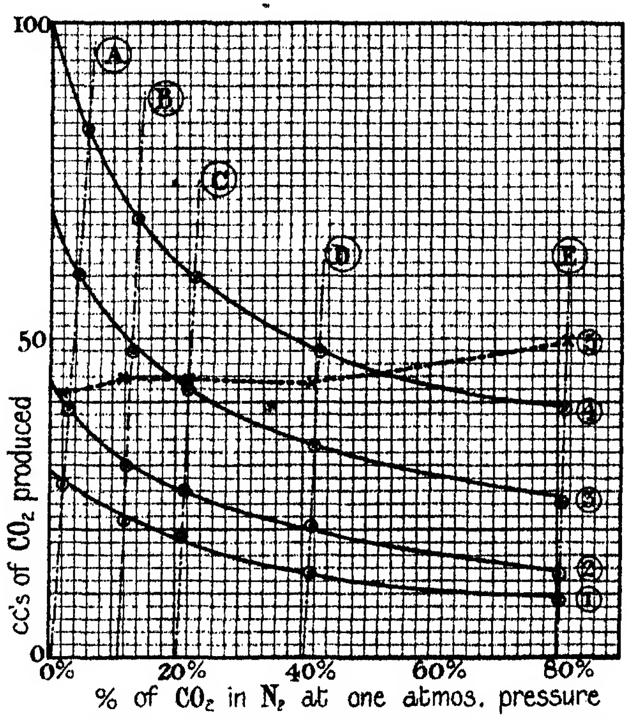

FIG. 1.—The retarding effect of carbon dioxide upon anaërobic CO_2 production, and the recovery after the removal of CO_2 concentrations. (See Table IV.)

1, 2, 3, 4—Total CO_2 production while under influence of CO_2 concentrations after 19, 31, 51, 90 hours respectively.

5—Total CO_2 production during 47 hours after removal of CO_2 concentrations.

A, B, C, D, E—Series of readings from five parallel experiments.

It is important to know how far the action of CO_2 upon anaërobic CO_2 production in these experiments is a constant retarding effect, and not merely confined to the first few hours. In fig. 2 the same results (Table IV) are given in different form, the total CO_2 evolved being plotted against time for two concentrations of CO_2, 0 and 20 per cent. (the values for these constant concentrations being taken from fig. 1). From these curves the curves for rate of CO_2 production plotted against time are deduced, and show at once that the depressant action of CO_2 is a continuous effect. In this case the average depressant effect of CO_2 during the whole 90 hours of experiments is 0·6, taking the control as 1. Both the first and the second 45 hours

of this period, if taken separately, also give 0·6 in either case as the average value for the depressant action of CO_2. Figs. 1 and 2 also show the return to equal rates of CO_2 evolution in the experiments of this series after the removal of the various depressant concentrations of CO_2.

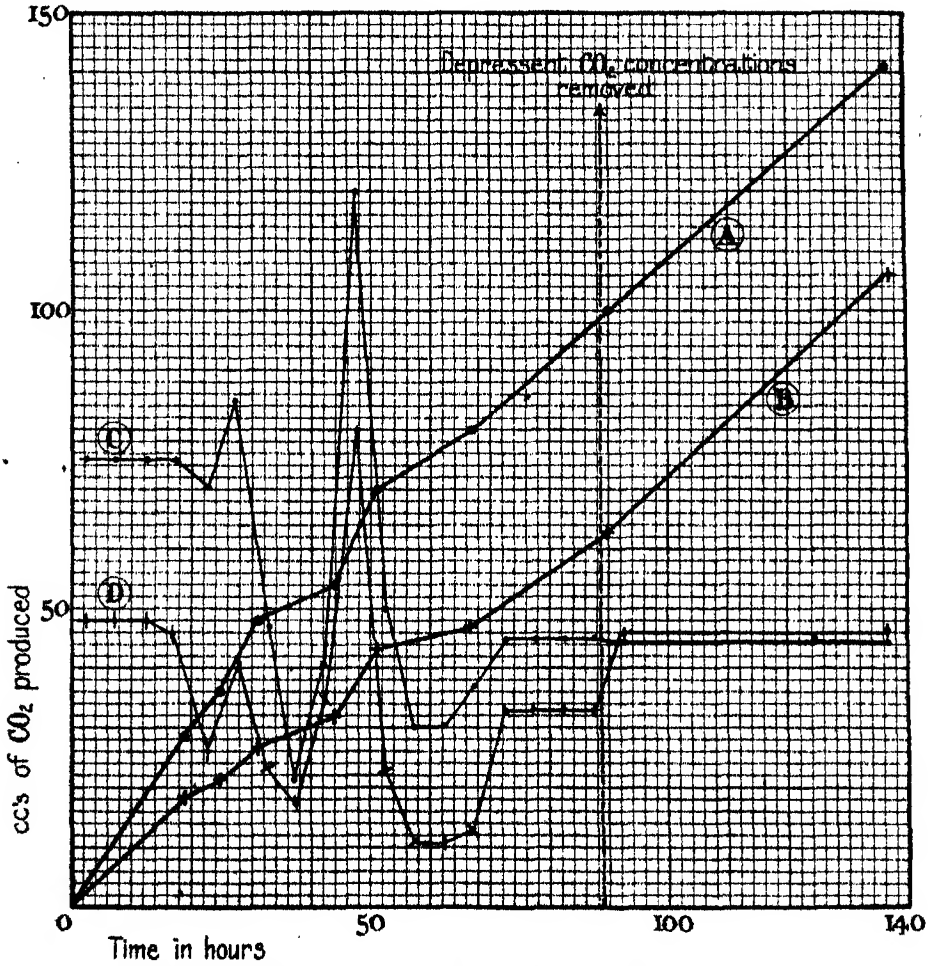

FIG. 2.—The retarding effect of carbon dioxide upon anaërobic CO_2 production and the recovery after the removal of CO_2 concentrations. (See Table IV and fig. 1.)

Curves A and B—Total CO_2 produced in the presence of 0 per cent. CO_2 and 20 per cent. CO_2 respectively.

Curves C and D—Rate of CO_2 production in the presence of 0 per cent. CO_2 and 20 per cent. CO_2 respectively [cubic centimetres per 5 hours magnified 20 times]. The fluctuations are due to laboratory temperature changes.

The effect of CO_2 appearing thus to be constant, we must be able to express in a general formula the retarding effect of different concentrations of CO_2.

Table VI.

	CO_2 required to reduce anaërobic respiration from 1 in pure nitrogen to				
	0·8.	0·6.	0·5.	0·4.	0·35.
	per cent.	per cent.	per cent.	per cent.	per cent.
90 hours curve	7	23	38	72	100
51 ,, ...	7	23	36	62	85
31 ,, ...	8	23	38	55	74
19 ,, ...	8	24	35	56	74
Average	75	23·3	36·75	60·75	83·2

Table VI, derived from the results plotted in fig. 1, gives the fractional depression of CO_2 production (taking that in absence of CO_2 = unity) for different concentrations of CO_2. This Table shows the results for all the four time curves in fig. 1 to be fairly concordant. These average values, supported by average values similarly obtained from other sets of experiments, are plotted in fig. 3, which thus represents for our experiments the general expression for the depressant action of CO_2 upon anaërobic CO_2 production.

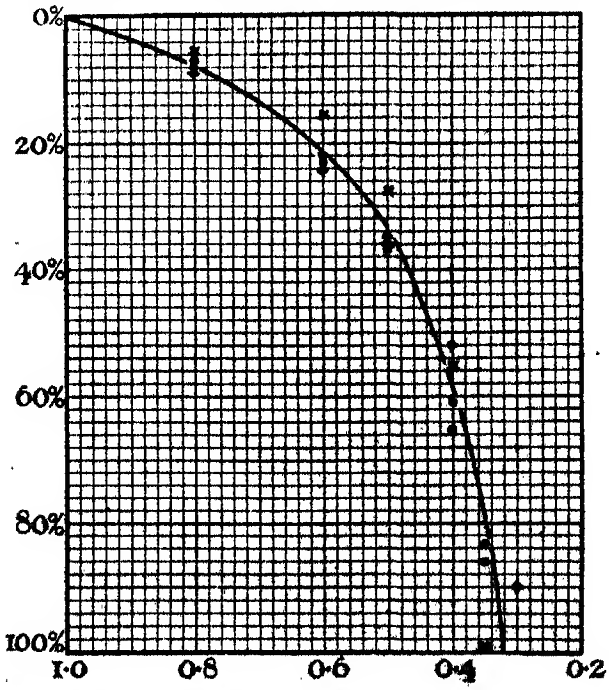

Fig. 3.—General expression for depressant action of carbon dioxide upon anaërobic CO_2 production.

In a consideration of this curve (fig. 3) the fact stands out that the regular retardation of CO_2 production does not appear to be towards zero. The simplest interpretation of this, perhaps, is to assume two processes concerned, one of which is regularly retarded to inhibition, the other being differently or not at all affected. This assumption is in line with our present knowledge with regard to "floating" and "protoplasmic" respiration. It is possible that only floating respiration as opposed to protoplasmic respiration is suppressed by carbon dioxide. The results of experiments described in a following section lend support to this view. It is clear in this case that until the magnitude of the protoplasmic respiration is known we cannot determine the base line, and therefore the exact form of the curve expressing the depression of floating respiration considered alone. The statement may be made at present as a useful formula that *up to about* 50 *per cent.* CO_2 *the depression of* CO_2 *production is roughly proportional to the square root of the* CO_2 *concentration.*

Section IV.—*The Influence of Carbon Dioxide upon Normal Respiration in the Presence of Oxygen (in Seeds).*

Experiments are next described which were directed to determine whether a similar depression is produced in the case of aërobic respiration under the influence of increased concentrations of carbon dioxide.

The method adopted in the following experiments was the same as that already described, save that 20 per cent. of oxygen was always present. The amount of respiration was measured both by oxygen intake and CO_2 output estimated from analyses, giving the respiratory quotient. The percentage of oxygen in the atmosphere, it will be seen, must decrease throughout the experiments. This has not appeared, however, to distort the results obtained. As has been shown by Stich* and others, the percentage of oxygen must be greatly decreased before the rate of respiration is affected. The experiments were terminated before the percentage of oxygen had fallen to such a degree as to limit respiration in this way.

The following Tables give the results:—

* O_2 was decreased to 3 per cent. before any effect was observed with seedlings.

Table VII.—Showing the Retarding Influence of Increased Concentrations of Carbon Dioxide upon the Rate of Normal Respiration in Germinating White Mustard Seeds, measured by CO_2 Production and Oxygen Consumption.*

	Concentrations of carbon dioxide initially present in atmospheres.					
	0 per cent.	10 per cent.	20 per cent.	30 per cent.	40 per cent.	80 per cent.
After 14 hrs. $\frac{\text{c.c. } CO_2 \text{ gain}}{\text{c.c. } O_2 \text{ loss}}$	$\frac{58}{71}$	$\frac{48}{57}$	$\frac{38}{49}$	$\frac{33}{45}$	$\frac{26}{38}$	$\frac{17}{32}$
Respiratory quotient	0·82	0·84	0·77	0·73	0·69	0·53
After 40 hrs. $\frac{\text{c.c. } CO_2 \text{ gain}}{\text{c.c. } O_2 \text{ loss}}$	$\frac{173}{197}$	$\frac{158}{185}$	$\frac{96}{122}$	$\frac{75}{104}$	$\frac{61}{97}$	$\frac{41}{90}$
Respiratory quotient	0·87	0·85	0·75	0·72	0·63	0·45

Conducted in dim diffuse light. 20 per cent. oxygen present initially in each experiment. 15 grm. of seed set dry on 50 c.c. damp sand and 10 c.c. tap water in each experiment. Results obtained from analyses.

Temperature of experiments 25·5° C., by thermostat.

Table VIII.—Showing the Retarding Influence of Increased Concentrations of Carbon Dioxide upon the Rate of Normal Respiration in Green Peas, Measured by CO_2 Production and Oxygen Consumption.

	Concentrations of carbon dioxide initially present in atmospheres.		
	0 per cent.	10 per cent.	25 per cent.
After 24 hours $\frac{\text{cc. } CO_2 \text{ formed}}{\text{cc. } O_2 \text{ loss}}$	$\frac{69}{66} = 1·05$	$\frac{41}{50} = 0·85$	$\frac{37}{45} = 0·85$
After 48 hours $\frac{\text{cc. } CO_2 \text{ formed}}{\text{cc. } O_2 \text{ loss}}$	$\frac{99}{92} = 1·07$	$\frac{75}{85} = 0·88$	$\frac{61}{72} = 0·84$

Conducted in dim diffuse light. In each experiment, 15 fully swelled green peas, weight 7·6 grm., removed from testas immediately before. Results obtained from analyses.

Temperature of experiment 25·5° C., by thermostat.

The above experiments show that the rate of normal respiration in the presence of excess of oxygen is retarded by the presence of carbon dioxide. This is a remarkable result. The values obtained in the experiments of Table VII when plotted give curves similar to those obtained in anaërobic

* The seeds used in this and other similar experiments with oxygen present obeyed the laws of carbon dioxide inhibition already demonstrated in Parts I and II of these researches.

conditions. The depressant action of CO_2 up to 50 per cent. varies roughly with the square root of the concentration. Sufficient work along this line has not yet been done to allow us to draw any definite quantitative conclusions. The general result, however, seems of importance, since in much experimental work upon respiration there must have been present sufficient CO_2 in the respiring tissues to depress the function appreciably and give misleading evaluations of the respiration. They have further a significant bearing upon the hypothesis of Crocker and others already referred to, which imputes dormancy when it occurs in certain moist seeds to lack of oxygen, on the grounds that their respiration is found to be reduced by the presence of the testa, the removal of which causes a rise in the rate of respiration and immediate germination. For, as has been shown, the CO_2 content of such seeds—in which, even in the absence of oxygen, CO_2 production would proceed anaërobically—will be raised by the presence of the testa limiting gaseous diffusion, and for this reason alone, even in excess of oxygen, the respiration would be reduced, as was found in the experiments of these workers.

Section V.—*The Influence of Carbon Dioxide upon Respiration (Aërobic and Anaërobic) in Leaves. Floating and Starvation Respiration.*

It has been pointed out that while carbon dioxide regularly retards respiration it does not, even in the highest concentration used, completely inhibit it. An interpretation of this result has been suggested above on the basis of Blackman's distinction between "protoplasmic" and "floating" respiration.

Blackman has shown that in the general respiration of a plant tissue we have at least two quite distinct types of respiration proceeding simultaneously: in the first place, a floating respiration, involving essentially an oxidation of carbohydrate or fat to carbon dioxide and water; secondly, a permanent substratum of "protoplasmic" respiration which is the necessary minimum of life. This is revealed, acting alone, in conditions of starvation.

Seeds cannot be reduced to a condition of starvation. Leaves have been used, therefore, in order to find if any difference exists between the action of CO_2 upon floating and carbohydrate respiration respectively. Most leaves can be quickly reduced to a starvation condition in the absence of light, and then show the typical protoplasmic respiration of Blackman.

The same general method was used here as already described, except that seeds were now replaced by cut leaves in the flasks. Several difficulties were encountered, the disturbance of cutting the leaf, the rapidity of starch disappearance, which renders it difficult to maintain leaves fully fed for the desired period of time, the variable nature of the material with regard to

age, water-content, stomatal aperture, and the consequent uncertainty in obtaining parallel lots such as are required for this method. Owing to these difficulties the results obtained were not so regular as in the case of seeds. It appeared, however, that when "starch" leaves were used a retardation under the influence of CO_2 occurred for a short period, but passed away as time went on. The leaves were, of course, kept in the dark during these experiments. The passing of the CO_2 retardation would correspond with the onset of starvation. When starved leaves were used, on the other hand, the same retardation was not observed. Both anaërobic and normal respiration were tested. In order to obtain the effect of CO_2 concentrations upon floating respiration alone equal lots of starved and starch leaves were set up under equal conditions for the desired concentration of CO_2. The difference between the respiration of the "starch" leaves and the starved leaves was taken as the value of the "floating" respiration. This subtraction method of determining the floating respiration is used tentatively. It gives, however, a definite result with regard to the effect of CO_2 upon floating respiration in agreement with our hypothesis. The fact must be borne in mind that this subtraction method depends on the assumption that the nature and amount of the protoplasmic respiration are the same in conditions of starvation and in conditions of full supply. Further results are awaited here.

Table IX.—Showing the Retarding Effect of Carbon Dioxide upon the Anaërobic CO_2 Production of "Starch" Leaves (Cherry Laurel), disappearing as starvation sets in and completely absent on the fifth day.

Initial CO_2 in N_2.	Anaërobic CO_2 production in successive periods.		
	1st day (18 hours).	2nd–4th day (18th–86th hour).	5th day (86th–108th hour).
per cent.	c.c.	c.c.	c.c.
0	25	98	12
10	17	85	11
20	12	85	15
50	14	79	—

Conducted in dark room. Whole leaves, 17 grm., 4 hours after cutting in each experiment. Cut at 8 p.m. Temperature of dark room 16·5–12·5° C. CO_2 production estimated from increased pressures.

Table X.—Effect of Carbon Dioxide upon Floating Respiration (Anaërobic) obtained by Subtraction Method.

Concentrations of CO_2 in N_2.	Anaërobic CO_2 produced (c.c. per 10 grm. per 10 hours).		
	By starch leaves.	By starved leaves.	By floating respiration (by subtraction).
per cent.			
0	20	7·8	12·2
35	8·8	8·4	0·4
85	7·1	5·9	1·2

Conducted in dark. 15 grm. of whole Cherry Laurel leaves in each experiment. Starch leaves kept after cutting five days under artificial illumination, petioles in H_2O. Starved leaves kept after cutting five days in dark, petioles in H_2O.

Temperature 25° C. Time 46 hours (second and third day after setting).

Table XI.—Effect of Carbon Dioxide upon Floating Respiration (Aërobic) obtained by Subtraction Method.

Concentration of CO_2 present.	CO_2 produced in normal respiration (c.c. CO_2 per 10 grm. per 10 hours).		
	By starch leaves.	By starved leaves.	By floating respiration (by subtraction).
per cent.			
10	18·5	6·7	11·8
25	15·7	7·9	7·8
40	16·4	10·4	6·0
60	15·8	12·4	3·4

Conducted in the dark. Ten whole Lilac leaves, 8 grm., in each experiment. Starch leaves, newly cut after 12 hours' sunshine, showed abundant starch. Starved leaves, newly cut from shoots kept previously six days in dark, showed complete absence of starch.

Temperature of experiments 20° C. Time 75 hours. Estimation by analysis.

These results indicate that floating respiration may be retarded to practical inhibition under the action of carbon dioxide. This is the case, moreover, both in the presence and in the absence of oxygen. Upon protoplasmic respiration, on the other hand, the action of carbon dioxide is shown to be of a different nature. The tentative conclusions reached can, therefore, be stated as follows:—(1) That the distinction drawn by Blackman between floating respiration and protoplasmic respiration is confirmed. (2) That the retarding and inhibiting action of carbon dioxide upon the general respiration of plant tissues (most marked in the case of seeds and seedlings so far as these experiments have as yet gone) is an effect, in the main, only upon the floating respiration and is in the nature of a direct action inhibiting it.

Section VI.—*Further Light upon the Chemical Mechanism of Respiration in Plants.*

The results of these researches bear directly upon the problem of the nature of the respiratory processes in plants; and especially upon the question as to whether the processes involved in the anaërobic CO_2 production which is induced in plants in the absence of oxygen, are not also normally the first processes in ordinary aërobic respiration.

Two very definite effects of carbon dioxide have been demonstrated:—

(1) An effect of increased concentrations of CO_2 in tissues upon anaërobic respiration, decreasing the rate of CO_2 production in direct relation to the amount of CO_2 in the tissues.

(2) An effect of increased concentrations of CO_2 in tissues upon normal respiration, decreasing the rate of this process also in direct relation to the amount of CO_2 in the tissues.

The similarity of these effects of carbon dioxide upon two processes which are different in their nature, the one producing CO_2 by molecular splitting, the other by direct oxidation, naturally suggests at once two possibilities. Either (1) the two processes are genetically connected, so that by limiting the precursor the second process is naturally and automatically limited also; or (2) while the two processes are not genetically connected, the similar effect produced on both under the influence of carbon dioxide is to be explained by an action of carbon dioxide upon the medium in which they both occur. Thus it is possible that a change in the permeability of protoplasm under the influence of carbon dioxide might decrease equally the rate of two reactions occurring in the cell but otherwise in no way connected with each other.

The fact that carbon dioxide appears to act in its retarding effect mainly upon the processes of floating respiration and not upon those of protoplasmic or starvation respiration inclines us to the first of these possibilities. Experiments were devised to test this question.

It is necessary, however, clearly to set out in the first place the hypothesis which we desire to test. It may be stated in sequence as follows:—(1) An anaërobic splitting of carbohydrate into some easily oxidisable substance and carbon dioxide, occurring in the initial stages of anaërobic respiration, is always the first process in normal respiration. (2) The rate of this first process producing easily oxidisable substances acts as a limiting factor upon the amount of the succeeding process, in which these substances are oxidised somehow by the oxygen of the atmosphere, oxygen being always in excess in normal respiration and the oxidisable substances being thus always completely removed. (3) Carbon dioxide retards the rate of the first process, that is the

anaërobic splitting process, only, but in so doing automatically retards also the amount of the following oxidations even in the presence of excess of oxygen.

On this hypothesis, if the amount of available oxygen be decreased till it is no longer present in excess, but in deficiency, so that the oxidisable substances produced in the first process accumulate faster than they are removed by oxidation, then the rate of the first splitting process will no longer be the limiting factor upon oxidation. In such conditions, if carbon dioxide has no effect upon the oxidation process, as we suppose, the amount of carbon dioxide present, while still retarding the first process, should now have no effect upon the amount of oxygen consumed.

In the following experiments peas covered with thick water films in order to reduce gaseous exchange were allowed to respire in the presence of 20 per cent. of oxygen. In this condition a large amount of CO_2 was produced anaërobically in addition to that accounted for by the loss of oxygen, showing clearly on our assumption that the available oxygen was present in an amount quite insufficient to remove the oxidisable substances as fast as they were produced in the first anaërobic splitting process.

As the following Table of results shows, carbon dioxide had not, under these conditions, any definite influence upon the amount of oxygen consumed.

Table XII.—Showing that if Oxygen is present in deficiency, increased concentrations of Carbon Dioxide, while still retarding the rate of Anaërobic CO_2 production, have now no effect in reducing the amount of Oxygen taken in.

Initial CO_2 in atmospheres N_2.	Oxygen consumed.	Anaërobic CO_2 production.
per cent.		
0	26	104
10	27	87
20	23	80
40	28	44

In each experiment 25 peas, dry weight 8 grm., with sufficient water to form adequate films over them when fully swelled. The flasks were shaken at intervals during the experiments.

The results shown in the Table were obtained after the experiments had been running 90 hours. The percentage of oxygen, initially 20 per cent., had fallen only to about 17 per cent. in each experiment.

The peas used were sterilised with 1/500 bromine for 30 minutes, and the flasks with weaker bromine for a longer period.

Temperature of the experiments was that of laboratory, 16° C. (average).

The increase in volume of the gases in the flasks during the experiments, as calculated from manometer readings, was taken as a measure of anaërobic respiration sufficient to demonstrate clearly the point that carbon dioxide retards anaërobic CO_2 production in these experiments.

From the foregoing Table, then, it appears that, when oxygen is not present in excess but in deficiency, a rise in the percentage of carbon dioxide in the atmosphere produces no corresponding fall in the oxygen consumption. This is in direct contrast to the descending series of figures representing the oxygen consumption when oxygen was present in excess.

This result strongly supports the first of the two hypotheses which have been put forward above. For, as the above results show, we can reduce the amount of available oxygen normally present in excess till a point is reached beyond which the rate of the first or splitting process producing oxidisable substances is no longer the factor limiting oxidation, but oxygen itself becomes instead the limiting factor; and in this case carbon dioxide has no longer any effect in reducing the amount of oxidation.

In short, the results thus obtained appear to offer considerable support to the general theory as to the genetic relation between anaërobic and aërobic respiration set out in full above.

Section VII.—*Carbon Dioxide Narcosis.*

In these researches, we have been concerned with the narcosis due to carbon dioxide which becomes apparent by retardation and inhibition of growth. The universal presence of CO_2 in living tissues makes it important to know the details of its action. At present it is, perhaps, best to consider the effects of CO_2 apart and not as the basis for generalisations as to the nature of narcosis. We have obtained, so far, the definite result that carbon dioxide reduces both the amount of anaërobic respiration and the amount of aërobic or normal respiration to a marked degree in plant tissues. Further, that of the two types of respiration included under these heads, demonstrated by Blackman and others, namely, floating respiration and protoplasmic respiration, it is the former only which is depressed, and that this may be retarded to practical inhibition under the action of CO_2.

It seems of importance to determine in the sequel what effect the membranes of cells of various types and various tissues may have upon the tensions of oxygen and carbon dioxide actually within the cytoplasm, effects for instance, such as are indicated in Loeb's experiments upon artificial fertilisation in which the destruction of a cortical layer in the cell was found to increase largely the rate of oxidation. R. Lillie has put forward the hypothesis that the cortical layer of the unfertilised egg prevents the diffusion of CO_2 from the egg and that this CO_2 inhibits oxidation. Further, it must be determined what effects oxygen and carbon dioxide themselves may have in changing the general permeability of cell membranes, such as, for instance, are indicated in researches like those of Osterhout and Lepeschkin in plant tissues, and those

of Ohrwall, Benedicenti and Treves, Straub, Lovatt Evans, Starling and Jerusalem, and Starling and Kaya in muscular tissue.

Section VIII.—*Conclusions.*

The results obtained may now be summarised. That this may be done more effectively it will be well to recall briefly the main results obtained in Parts I and II of these researches, which were as follows:—

1. The resting stage of the moist seed is primarily a phase of narcosis induced by the action of carbon dioxide.

2. Both the arrested development in the case of the moist maturing seed and the widely occurring phenomenon of delayed germination in the case of the moist resting seed, are related to an inhibitory partial pressure of carbon dioxide in the tissues of the embryo.

3. Germination is related to a lowering of this inhibitory partial pressure of carbon dioxide in the tissues.

4. The inhibitory value of a given carbon dioxide pressure diminishes with a rise of temperature.

5. The inhibitory value of a given carbon dioxide pressure diminishes with a rise of oxygen pressure.

In Part III the investigation has been extended to plant tissues in general, in order to determine the mechanism of this narcosis. The influence of carbon dioxide upon respiration has been first studied, in view of the fact that respiration appears closely connected with growth by cell division. The conclusions reached may be summarised as follows:—

1. The rate of anaërobic CO_2 production in plant tissues is depressed by carbon dioxide.

2. This depression of CO_2 production is not due to permanent disorganisation. It passes away as soon as the depressant concentration of CO_2 is removed.

3. Quantitatively the degree of depression at the temperatures used appears to be proportional to the square root of the concentration of CO_2, over a range from 0 to 50 per cent. CO_2 at one atmosphere pressure. Above 50 per cent. the effect of increasing the concentrations becomes gradually less marked.

4. This depressant action of carbon dioxide is not limited to anaërobic CO_2 production, but occurs also in aërobic respiration in the presence of oxygen.

5. The depression of aërobic respiration under carbon dioxide is shown when measured either by O_2 consumption or CO_2 production.

6. Where oxygen is in deficiency so that some degree of anaërobic CO_2 production occurs, it is then found that carbon dioxide has no retarding effect on oxidation.

7. A quantitative relation exists between the concentration of carbon dioxide and the depression of aërobic respiration of the same order as in the case of anaërobic CO_2 production.

8. These results are taken as evidence that anaërobic and aërobic CO_2 production are processes genetically connected in normal respiration, and that the rate of the anaërobic process acts as the limiting factor in normal respiration.

9. Of the two types of respiration demonstrated by Blackman and others, namely, floating respiration and protoplasmic respiration, it is the former only which is depressed by the retarding action of carbon dioxide.

10. The main conclusion to be drawn from these results with regard to the inhibitory action of CO_2 upon growth is that a marked reduction of respiration is involved in the mechanism of CO_2 narcosis.

In conclusion I wish to thank Dr. F. F. Blackman, who has continually given me most valuable help in consultation during these researches, and also Prof. V. H. Blackman, for whose criticism and advice I am much indebted.

The Growth of the Body in Man.—The Relationship between the Body-weight and the Body-length (Stem-length).

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(Communicated by Prof. C. S. Sherrington, F.R.S. Received October 15, 1915.)

(From the Department of Pathology, University of Oxford.)

A number of investigations carried out in recent years by Prof. Dreyer and myself have indicated the existence of several previously unknown relationships which hold throughout a given species of animals between functions of the body-weight and various other ascertained measurements.*

In connection with work of a similar character on which we are engaged, I undertook some years ago the collection and examination of data bearing on the growth of Man. This work is still incomplete, but certain results of interest have already emerged.

The present communication deals with the relationship between the length of the human body and its weight during the period of growth. By the term "length" of the body is here meant the length of the stem of the body constituted by the head, neck and trunk; that is to say the distance from the top of the head to the line joining the ischial tuberosities, or stem-length as it may be termed.

This measurement corresponds to the body-length of animals and was chosen as the first object for investigation in order that any results which were obtained in Man might be comparable with those obtained in other species.

Method of Measurement.

Length.—The length is measured by seating the subject on the floor or on a low table (not a chair) with the back against the wall. Care is taken to see that the sacrum is in contact with the wall, and the legs somewhat drawn up so that the individual sits fairly upon his ischial tuberosities. Under these conditions the height of the top of the head gives a true measurement of the length of the body, and one which is constant and incapable of variation by the subject.

If a chair or other form of seat be employed in taking this measurement

* Dreyer, Georges, and Walker, E. W. Ainley, "The Determination of the Minimal Lethal Dose of various Toxic Substances and its Relationship to the Body-weight in Warm-blooded Animals, together with Considerations bearing on the Dosage of Drugs," 'Roy. Soc. Proc.,' B, vol. 87, p. 319 (1914), and references to literature contained therein.

the individual can by "sitting low" or "sitting high" produce at will a variation of as much as 3 or more per cent. But, since a subject conscious that he is being measured for height tends naturally to produce a full measurement, it will be found that he intentionally "sits up," straightening the spine, tilting the pelvis forward, and rests on the contracted muscles of the thighs and buttocks instead of on his ischial tuberosities. The apparent length—"sitting height," as it has been termed—is thus increased by between 2 and 3 per cent. above the measurement of length taken in the manner already described. Accordingly measurements taken on a seat require to be corrected down appropriately before they can be treated as comparable with the measurement of body-length in infants or animals.

In infants too young to sit the measurement of length is carried out as follows. Two pairs of hands are required. A table is brought up against the wall and the infant is laid upon the table on its back so that the head carefully held in position just touches the wall. The pelvis is held down upon the table, the thighs flexed on the trunk, and a vertical flat piece of board is brought up against the nates. The distance between the board and the wall gives the length of the body.

Weight.—The weight of the body is, of course, the weight without clothes.

Data and Calculations.

The data employed in the present investigation consist in part of measurements made by myself; and in part of measurements kindly obtained for me by Mr. G. Haynes at the Oxford Preparatory School; by Mr. Carter at the New College (Oxford) Choir School; and by Miss Poulton at Malvern College for Girls. To each of them I am very greatly indebted for the care and accuracy with which my directions for measurement were carried out. All the measurements in infants and very young children, as well as some in older children, were made by myself, Mr. Haynes supplied the data for 94 boys, Mr. Carter those for 19 boys, and Miss Poulton those for 56 girls.

The subjects were all healthy normal individuals, well nourished and living under favourable conditions. Their average weight and height for each year of age are above those usually given in tables for the general population.

In addition to the data already mentioned I had at my disposal, by the kindness of Dr. E. H. J. Schuster, measurements made by him on 1500 undergraduates at Oxford.

These data have formed the basis of a series of calculations whose result has been to show that the length of the body (stem-length) in man can

correctly be expressed as a function of the body-weight, and conforms to the formula

$$l = k\mathrm{W}^n,$$

where l is the length of the body (stem-length) in millimetres, W the weight (without clothes) in grammes, k a constant, and n a power of the approximate value $\frac{1}{3}$. The evidence on which this conclusion is based will now be presented.

Males.

Table I (not printed, but preserved for reference in the archives of the Royal Society) contains a full record of the data for boys, together with the calculated value of the length constant k for each individual, deduced from the formula $k = \mathrm{W}^n/l$, where n has the value 0·33 as determined in Table II.

In Table II the boys are grouped according to weight in twenty groups. The average body-weight and average body-length for each group is set out, and the figures in the various columns are calculated for each group from the average body-weight and body-length of the group. The body-weights of the groups cover a range in weight from 8168 grm. to 51,480 grm. and show a more than six-fold increase from the lightest group to the heaviest.

In the first instance the "best n" for these groups in the formula $l = k\mathrm{W}^n$ was ascertained graphically to lie in the neighbourhood of 0·3. The precise values of n and k were then determined by trial from the formula $\log k = \log l - n \log \mathrm{W}$. The "best n" was thus found to have the value 0·33 while the value 0·32 for n is nearly as good.

The values of k for the groups are shown in the columns for length constant calculated and are seen to be free from periodicity. They give an average value for k of 23·23 when n is 0·33, and a value of 25·73 when n is 0·32.

Substituting these values of n and k in the formula $l = k\mathrm{W}^n$ the theoretical value of l is calculated for each group in the appropriate column. The observed values of l are in good agreement with these calculated values and show an average deviation from them of only 1·32 per cent.

If account be taken of the number of individuals in each group the average deviation becomes 1·10 per cent. when n is 0·33, and 1·14 per cent. when n is 0·32. The mean deviation calculated by the method of least squares is, under the same circumstances, 1·48 per cent. when the best n (0·33) is used, and 1·50 per cent. when n is given the value 0·32.

It follows, therefore, that the stem-length of boys conforms to the formula

Table II.—Boys Grouped: Data and Calculations.

No. of group.	No. of individuals in group.	Average body weight, W.	Average body-length (stem-length) observed, l.	Body-length as a percentage of body-weight.	Length constant calculated. $k = W^n/l$. $n = 0·32$.	Length constant calculated. $k = W^n/l$. $n = 0·33$.	Body-length calculated. $l = kW^n$. $n = 0·32$. $k = 25·73$.	Difference between body-length calculated and observed. $n = 0·32$. $k = 25·73$.	Body-length calculated. $l = kW^n$. $n = 0·33$. $k = 23·23$.	Difference between body-length calculated and observed. $n = 0·33$. $k = 23·23$.
		grm.	mm.				mm.	per cent.	mm.	per cent.
1	1	51,480	800·0	1·55	24·85	22·29	828·1	3·39	833·7	4·04
2	7	45,340	779·6	1·72	25·22	22·66	796·2	1·96	799·3	2·46
3	5	42,690	775·8	1·82	25·59	23·00	780·0	0·54	783·6	1·00
4	10	40,890	773·9	1·89	25·88	23·27	769·3	0·60	772·7	0·16
5	4	39,520	764·5	1·93	25·84	23·26	761·0	0·46	763·8	0·09
6	7	37,620	743·0	1·98	25·52	22·97	749·1	0·81	751·6	1·14
7	8	36,300	741·8	2·05	25·79	23·22	739·9	0·26	742·2	0·05
8	14	35,220	738·6	2·10	25·92	23·34	733·3	0·72	735·3	0·45
9	13	34,010	732·0	2·15	25·96	23·40	725·2	0·94	726·9	0·70
10	10	32,910	729·5	2·22	26·15	23·57	717·6	1·66	719·1	1·45
11	10	31,850	725·8	2·28	26·29	23·70	710·3	2·18	711·4	2·02
12	11	31,030	705·1	2·27	25·75	23·23	704·4	0·10	705·3	0·03
13	10	28,150	687·3	2·44	25·90	23·38	682·6	0·69	682·9	0·64
14	10	25,740	675·1	2·62	26·18	23·65	663·4	1·76	663·1	1·81
15	6	22,360	656·7	2·94	26·65	24·11	634·2	3·55	633·0	3·74
16	3	20,040	607·0	3·03	25·51	23·15	612·4	0·88	610·5	0·57
17	6	17,960	591·3	3·29	25·72	23·32	591·3	0·00	588·9	0·41
18	3	14,670	540·7	3·69	25·27	22·97	550·4	1·76	547·0	1·15
19	4	12,250	502·8	4·10	24·73	22·51	523·1	3·88	519·0	3·12
20	5	8,168	460·4	5·64	25·78	23·56	459·5	0·20	450·0	1·41
	147	Average			25·73	23·23	—	1·32 (1·317)	—	1·32 (1·322)
		Average, taking into account number of individuals in each group						1·14	—	1·10
		Mean deviation, taking into account number of individuals in each group ...						1·50	—	1·48

$l = kW^n$, where n has the value 0·33 and the value of k for the grouped boys is 23·23.

For the individual boys the average value of k is 23·33. Its greatest value in the series (25·27) exceeds the average value by 8·32 per cent., and its least value (21·08) falls below the average value by 9·69 per cent.

Using the average value of k (23·33) and the value 0·33 for n, the theoretical body-length of each individual has been calculated from the body-weight by means of the formula

$$l = 23{\cdot}33\, W^{0{\cdot}33}.$$

From consideration of space the figures have not been tabulated, but they show an average percentage difference between the calculated body-length and that actually observed for the individual boys of only 2·63 per cent., and a mean deviation calculated by the method of least squares of 3·378 per cent.

Accordingly it may be taken that the formula holds very satisfactorily for boys. In these boys, however, the ages do not exceed 15 years, nor the weights 51,000 grm. (8 stone 1 lb.). Hence before it can be assumed that the formula holds up to adult age some further evidence is desirable. This may be found in a study of the data provided by Dr. Schuster's measurements of undergraduates, along with certain measurements of undergraduates made by myself.

Dr. Schuster's data consist of the measurements of two series of undergraduates during their first year at the University, a first series of 1000 individuals and a second series of 500. They are presented in Tables III and IV as grouped by myself.

In dealing with these figures for grouped undergraduates it was necessary at the outset to apply *two corrections.* In the first place Schuster's measurement of body-length is the "sitting height" as measured on a fixed seat. But from observations which I made on a series of individuals measured for the purpose in a similar manner, and also in the manner described in this paper, it appeared that the sitting height by the former method is always greater by from 2 to 3 per cent. than the measurement of length on which the present investigation is based. Between the limits 2 and 3 per cent. the precise value of the excess measurement depends very much on how the individual seats himself, and on the degree to which he lifts himself on his thigh muscles as explained in the section on method of measurement.

Accordingly I have taken the mean of these limits (*i.e.* 2·5 per cent.) as the amount to be deducted from the average sitting heights to obtain the true value of the average body-lengths for the groups. The second correction

Table III.—Undergraduates—First Series—Data, Corrections, and Calculations.

Group.	No. in group.	Average body-weight partly clothed, w.	Average sitting height, λ.	Weight of part clothes calculated. $c = kw^n$, $n = 0·29$, $k = 69·63$. c.	Body weight corrected, W.	Body-length corrected. $l = 975\lambda/1000$ (stem-length) l.	Body-length as a percentage of body-weight.	Length constant calculated. $k = W^n/l$, $n = 0·33$.	Length constant calculated using "best n." $n = 0·20$.	Body-length calculated. $l = kW^n$. $n = 0·20$. $k = 98·22$.	Difference between body-length calculated and observed.
		grm.	mm.	grm.	grm.	mm.				mm.	per cent.
1	7	92,080	975	1916	90,160	951	1·05	22·03	97·07	962	1·14
2	40	84,820	971	1871	82,950	947	1·14	22·55	98·28	946	0·11
3	55	80,290	961	1838	78,450	937	1·19	22·73	98·35	936	0·11
4	77	77,570	955	1827	75,740	931	1·23	22·84	98·40	929	0·22
5	119	74,390	951	1801	72,590	927	1·28	23·07	98·83	921	0·65
6	161	71,220	939	1778	69,440	916	1·32	23·14	98·54	913	0·33
7	162	68,040	933	1755	66,280	910	1·37	23·33	98·99	905	0·55
8	151	64,410	925	1728	62,680	892	1·42	23·30	97·95	895	0·34
9	111	61,690	913	1706	59,980	890	1·48	23·59	98·58	887	0·34
10	65	58,970	900	1684	57,290	878	1·53	23·62	98·15	879	0·11
11	34	56,790	884	1657	54,130	862	1·59	23·63	97·45	869	0·81
12	18	51,710	876	1621	50,090	854	1·70	24·02	98·06	856	0·23
	1000				Average			(23·15)	98·22	—	0·41
					Average, taking into account number of individuals in each group						0·38
Average of groups					68,320	908					
Average of individuals					67,270	908					

Table IV.—Undergraduates—Second Series—Data, Corrections, and Calculations.

Group.	No. in group.	Average body-weight partly clothed, w.	Average sitting height, λ.	Weight of part clothes calculated. $c = kw^n$. $n = 0 \cdot 29$. $k = 69 \cdot 63$. c.	Body-weight corrected, W.	Body-length corrected. $l = 975\,\lambda/1000$ (*stem-length*), l.	Body-length as a percentage of body-weight.	Length constant calculated. $k = W^n/l$. $n = 0 \cdot 33$.	Length constant calculated using "best n." $n = 0 \cdot 19$.	Body-length calculated. $l = kW^n$. $n = 0 \cdot 19$. $k = 109 \cdot 9$.	Difference between body-length calculated and observed.
		grm.	mm.	grm.	grm.	mm.				mm.	per cent.
1	8	93,440	977	1924	91,520	955	1·04	21·97	109·0	963	0·83
2	14	85,280	970	1874	83,410	946	1·13	22·49	109·8	946	0·00
3	49	78,730	955	1831	76,900	931	1·21	22·73	109·8	932	0·11
4	50	74,390	954	1801	72,590	930	1·28	23·14	110·9	922	0·87
5	92	72,580	936	1788	70,790	913	1·29	22·91	109·4	918	0·54
6	83	68,040	935	1755	66,280	912	1·38	23·38	110·6	906	0·66
7	79	64,860	921	1731	63,130	898	1·42	23·40	110·0	898	0·00
8	62	61,690	909	1706	59,980	886	1·48	23·48	109·5	889	0·34
9	41	58,510	899	1680	56,830	877	1·54	23·66	109·5	880	0·34
10	22	54,490	893	1645	52,840	871	1·65	24·06	110·4	868	0·35
	500				Average			(23·12)	109·9	—	0·40
					Average, taking into account number of individuals in each group						0·33
Average of groups					69,430	912					
Average of individuals					67,020	907					

is a more complicated matter and concerns the weights. These are not true body-weights, but include the weight of a portion of the clothes, only the coat and boots having been removed in each case before weighing.

In order to arrive if possible at a reliable correction for the weight of this portion of clothing I obtained the weights clothed (partially) and unclothed, as well as other measurements, of 30 undergraduates who happened to be my pupils at the moment. These 30 men are grouped according to weight in Table V. From the average body-weight (part clothed) and the average weights of the part-clothes for the groups a formula for part-clothes was arrived at in the form $c = 69{\cdot}63\ w^{0{\cdot}29}$. This formula fits excellently, the average percentage difference between c calculated by this formula and c observed being only 0·50 per cent., and the mean deviation by the method of least squares 0·61 per cent.

As a further check for the part-clothes their weight was also expressed (see Table V) in percentage of the corresponding body-weight (partly clothed) and the percentage of body-weight (partly clothed) to be assigned to clothes for each group in Table III and Table IV was estimated by interpolation and extrapolation from this series. The values thus obtained were found to agree very satisfactorily with the weights for clothes calculated for the groups in these two Tables by the part-clothes formula. The latter were therefore employed in the Tables in question in order to arrive at the true (unclothed) body-weight for each group.

But the groups in Tables III and IV are not in any case groups which can be expected to exhibit the normal average relation between body-weight and body-length. They cannot be employed as groups in the calculation of a formula for the relationship between these measurements *as growth proceeds*, in the way in which the groups for boys have already been used, for this reason, that they represent a body of individuals measured *at a particular age*. They constitute a selected material, being all University freshmen of an age somewhere between 18 and 20 years, probably for the most part between 18 and 19 years, at the time of observation. Thus the heavier groups do not represent a further stage of growth of the lighter groups. But the whole material simply illustrates the range of measurement from little men to big men at a given age. Hence they differ from a natural growth series by lacking from the groups at the lighter end adolescent boys over 50,000 grm. in weight—say from 15 to 18 years of age—whose inclusion would diminish the average values of l in the lower groups. And they lack at the heavier end all more fully-grown young men of 20 years of age and upwards, whose inclusion would increase the average values of l in the upper groups.

Table V.—Relation between Body-weight (Partly Clothed) and Part Clothes—Data and Calculations.

No. of group.	No. of individuals in group.	Average body-weight partly clothed, w.	Average weight of part clothes, c.	Part clothes as a percentage of body-weight partly clothed.	Part clothes constant calculated. $k = w^n/c$ $n = 0 \cdot 29$.	Weight of part clothes calculated. $c = kw^n$. $n = 0 \cdot 29$. $k = 69 \cdot 63$.	Difference between weight of part clothes calculated and observed.	Average body-weight without clothes, W.	Average body-length (stem-length) observed, l.
		grm.	grm.				per cent.	grm.	mm.
1	4	86,240	1883	2·18	69·74	1880	0·11	84,360	922
2	6	77,540	1817	2·34	69·39	1823	0·33	75,720	934
3	4	70,680	1793	2·54	70·36	1774	1·07	68,890	915
4	4	67,900	1748	2·57	69·37	1754	0·34	66,150	901
5	6	64,180	1711	2·67	69·04	1726	0·87	62,470	872
6	6	58,830	1688	2·87	69·85	1683	0·30	57,140	894
	30	Average			69·63	—	0·50		
		Average, taking into account number of individuals in each group..............					0·50		
		Mean deviation, taking into account number of individuals in each group ...					0·61		
		Average of individuals						66,540	905

Accordingly the groups in Tables III and IV do not fit the formula for grouped boys, $l = 23{\cdot}23\ W^{0{\cdot}33}$. They give a series of values for the length constant as calculated from the expression $k = W^{0{\cdot}33}/l$, which increases regularly from a value of about 22 to a value of about 24 as the weight diminishes.

As a matter of fact the calculations summarised in Table III show that the 1000 undergraduates of the first series as grouped give a value for k of 98·22 with a "best n" of 0·20 and fit the formula $l = 98{\cdot}22\ W^{0{\cdot}20}$. *And the calculations summarised in Table IV for the 500 undergraduates of* the second series give the value 109·9 for k with a "best n" of 0·19.

These facts, however, do not mean that the body-length and body-weight of young men are differently related to each other from the same measurements in boys, and that their bodies are built on a different plan.

The discrepancy depends entirely on the fact that the individuals in question are selected individuals—selected for age. It is well brought out by comparing the figures for body-length as a percentage of body-weight in the heavier boys (Table II) with the corresponding figures for the lighter groups of undergraduates (Tables III and IV).

That it is due entirely to the fact of age-selection follows from the observation that precisely the same phenomenon appears if we select for age among the boys whose measurements have just yielded the formula $l = 23{\cdot}23W^{0{\cdot}33}$. This is seen to be the case in four instances in Table VI where the boys already dealt with have been selected for age, the ages chosen being 11 to 12, 10 to 11, 9 to 10, and 7 to 9 years respectively. In each case the individuals are grouped according to weight, and the value of k is calculated for each group from the average body-weight and average body-length of the group by means of the formula $k = W^{0{\cdot}33}/l$. It is at once evident that in every case by selecting for age and thus excluding from the lower half of each series the heavy boys of ages below the selected age, and from the upper half the lighter boys of ages more advanced, we produce a series of groups which is exactly comparable to the groups exhibited in Tables III and IV.

But though these groups of undergraduates cannot be employed as groups for the calculation of a growth formula, there remains a way in which their measurements can be employed to test the formula for males. This is by averaging the whole material of each series of observations so as to obtain an average individual. These averages are given in Table VII along with the averages of my own 30 undergraduates, and for each series the length constant is calculated from the expression $k = W^{0{\cdot}33}/l$. It happens by a curious chance that the three figures are identical. But the point of importance is

that the value of k thus calculated falls remarkably close to the value of k in the formula for boys, the figures being respectively 23·17 and 23·23.

Table VI.—Boys—Selected for Age—Data and Calculations.

Age.	Group.	No. in group.	Average body-weight, W.	Average body-length (stem-length), l.	Length constant calculated. $k = W^n/l$. $n = 0·33$.
			grm.	mm.	
11 to 12 years ...	1	3	40,050	754	22·83
	2	8	36,710	738	23·00
	3	7	34,410	735	23·40
	4	5	33,250	733	23·60
	5	2	32,200	732	23·82
10 to 11 years ...	1	4	35,360	729	22·48
	2	5	32,870	720	22·92
	3	5	31,530	712	23·33
	4	4	30,140	709	23·58
	5	4	28,130	697	23·71
9 to 10 years ...	1	2	32,210	715	23·27
	2	4	30,450	709	23·50
	3	3	27,770	695	23·74
	4	2	25,860	668	23·35
	5	2	22,680	666	24·33
7 to 9 years ...	1	2	31,870	703	22·96
	2	4	27,440	676	23·18
	3	4	25,040	670	23·69
	4	2	23,700	668	24·05

Accordingly it may fairly be concluded that the formula established for boys holds for young adults, and therefore accurately represents the relationship existing between the body-weight and the body-length in males throughout the period of growth from birth to early adult age.

Table VII.—Length Constant calculated for Average Undergraduates.

Average of individuals.	Average body-weight, W.	Average body-length, l.	Length constant calculated. $k = W^n/l$. $n = 0·33$.
	grm.	mm.	
First series of 1000 undergraduates	67,270	908	23·17
Second series of 500 undergraduates......	67,020	907	23·17
Own series of 30 undergraduates	66,540	905	23·17

In view of the fact that I have been unable to state with confidence the correction for l in Schuster's undergraduates more precisely than that it

represents a diminution of the observed sitting height by somewhere between 2 and 3 per cent. I have prepared a Table (Table VIII) showing the variations in the calculated values of k when different corrections between these limits are applied to the sitting height, and also when no correction is made.

Table VIII.—Length Constant calculated with Various Corrections for l.

Average under-graduate.	Correction, 2·0 p.c.		Correction, 2·25 p.c.		Correction, 2·5 p.c.		Correction, 2·75 p.c.		Correction, 3·0 p.c.		No correction.	
	Value of		Value of		Value of		Value of		Value of		Value of	
	l.	k.	l.	k.	l.	k.	l.	k.	l.	k.	l.	k.
Series 1	912	23·27	910	23·21	908	23·17	905	23·09	903	23·04	931	23·75
Series 2	911	23·28	909	23·23	907	23·17	904	23·10	902	23·04	930	23·82

Females.

Table IX (not printed) contains a full record of the data for females, together with the calculated value of the length constant k for each individual deduced from the formula $k = W^n/l$ where n has the value 0·32 as determined in Table X.

In Table X the girls are grouped according to weight in 16 groups. The average body-weight and average body-length for each group is set out, and the figures in the various columns are calculated for each group from the average body-weight and body-length of the group. The body-weights of the groups cover a range in weight from 3834 to 76,430 grm. and show a 20-fold increase from the lightest group to the heaviest.

The "best n" for these groups in the formula

$$l = kW^n$$

has the value 0·32, while the value 0·33 is nearly as good. The values of k for the groups are shown in the columns for length constant calculated, and are seen to be free from periodicity. They give an average value for k of 25·60 when n is 0·32, and a value of 23·17 when n is 0·33.

Substituting these values of n and k in the formula $l = kW^n$ the theoretical value of l is calculated for each in the appropriate column. The observed values of l are in good agreement with these calculated values and show an average percentage deviation from them of only 1·41 per cent. when n is 0·32 (1·49 per cent. when n is 0·33).

Table X.—Girls Grouped—Data and Calculations.

No. of group.	No. of individuals in group.	Average body-weight, W.	Average body-length (stem-length) observed, l.	Body-length as a percentage of body-weight.	Length constant calculated. $k = W^n/l$. $n = 0.32$.	Length constant calculated. $k = W^n/l$. $n = 0.33$.	Body-length calculated. $l = kW^n$. $n = 0.32$. $k = 25.60$.	Difference between body-length calculated and observed. $n = 0.32$. $k = 25.60$.	Body-length calculated. $l = kW^n$. $n = 0.33$. $k = 23.17$.	Difference between body-length calculated and observed. $n = 0.33$. $k = 23.17$.
		grm.	mm.				mm.	per cent.	mm.	per cent.
1	1	76,430	940·0	1·23	25·72	22·99	935·3	0·46	947·1	0·75
2	7	61,030	857·9	1·41	25·24	22·61	870·2	1·41	879·2	2·42
3	10	54,250	847·1	1·56	25·88	23·20	837·9	1·10	845·9	0·14
4	10	53,660	831·2	1·55	25·48	22·85	835·1	0·47	842·8	1·38
5	9	49,390	797·2	1·61	25·11	22·54	813·2	1·92	820·0	2·73
6	8	46,380	795·4	1·71	25·55	22·95	796·9	0·19	803·2	1·10
7	8	41,000	770·7	1·90	25·75	23·16	766·1	0·60	771·1	0·05
8	4	36,990	740·5	2·00	25·57	23·01	741·3	0·11	745·4	0·66
9	5	30,800	724·8	2·39	26·68	24·17	695·3	4·24	697·9	3·85
10	5	24,200	659·6	2·73	26·09	23·59	647·1	1·93	647·9	1·81
11	4	20,170	609·3	3·02	25·55	23·13	610·5	0·20	610·2	0·15
12	4	15,720	559·8	3·56	25·42	23·08	563·7	0·69	562·0	0·39
13	4	10,420	485·3	4·66	25·14	22·92	494·2	1·80	490·7	1·10
14	3	7,876	466·0	5·92	26·34	24·09	451·9	3·12	447·4	4·16
15	2	5,602	391·5	6·99	24·73	22·69	405·2	3·38	399·8	2·08
16	2	3,834	355·5	9·27	25·35	23·34	358·9	0·95	352·8	1·05
	86	Average			25·60	23·17	—	1·41	—	1·49
		Average, taking into account number of individuals in each group						1·28	—	1·43
		Mean deviation, taking into account number of individuals in each group ...						1·59	—	1·87

If account be taken of the number of individuals in each group the average percentage deviation becomes 1·28 when n is 0·32, and 1·43 when n is 0·33. The mean deviation calculated by the method of least squares is, under the same circumstances, 1·59 per cent. when the "best n" (0·32) is used, and 1·87 per cent. when n is given the value 0·33.

It follows, therefore, that the stem-length of girls conforms to the formula $l = kW^n$, where n has the value of 0·32 and the value of k for the grouped girls is 25·60. Moreover, since the grouped girls range in weight from 3834 to 76,000 grm. (12 stone 5 lb.), and in age from two weeks to over 17 years, it may probably be taken that the formula holds for females generally throughout the period of growth from birth to adult age.

For the individual girls the average value of k is 25·58. Its greatest value in the series (28·68) exceeds the average value by 12·12 per cent., and its least value (22·58) falls below the average value by 11·73 per cent.

Using the average value of k (25·58) and the value 0·32 for n, the theoretical body-length of each individual has been calculated from the body-weight by means of the formula

$$l = 25{\cdot}58\,W^{0{\cdot}32}.$$

The figures thus obtained (which from consideration of space have not been tabulated) showed an average percentage difference between the calculated body-length and that actually observed for the individual girls of only 3·02 per cent., and a mean deviation (calculated by the method of least squares) of 4·149 per cent.

The Difference between the Sexes.

The formula for the grouped males has been shown to be

$$l = 23{\cdot}23\,W^{0{\cdot}33}.$$

That for the grouped females is $l = 25{\cdot}60W^{0{\cdot}32}$. If we desire to work in pounds avoirdupois and inches instead of grammes and millimetres, the formula for males becomes $l = 6{\cdot}91W^{0{\cdot}33}$, and that for females $l = 7{\cdot}14\,W^{0{\cdot}32}$.

Now, it has been noted in what has gone before that while the best n for males is 0·33 and the best n for females is 0·32, in each case the values 0·32 and 0·33 are nearly equally good (values on either side of these quantities being distinctly less good).

This fact at once suggested that in taking the power 0·33 for males and 0·32 for females, the sex difference was to some extent exaggerated, the true value of n lying in each case somewhere between 0·32 and 0·33, but nearer 0·32 for females and nearer 0·33 for males.

Accordingly, the "best n" was recalculated for each sex to three places of

decimals after regrouping the individuals in a small number of groups to diminish the labour of calculation.

Table XI exhibits the result obtained for males, Group 1 representing the 30 undergraduates measured by myself, and the remaining groups the boys in Table II.

The "best n" for males was found to have the value 0·329, with a value for k of 23·45, giving the formula $l = 23{\cdot}45\mathrm{W}^{0{\cdot}329}$.

Table XI.—Males re-grouped—Data and Calculations.

Group.	No. in group.	Average body-weight, W.	Average body-length (stem-length), l.	Length constant calculated. $k = \mathrm{W}^n/l$. $n = 0{\cdot}329$.	Body-length calculated. $l = k\mathrm{W}^n$. $n = 0{\cdot}329$. $k = 23{\cdot}45$.	Difference between body-length calculated and observed.
		grm.	mm.		mm.	per cent.
1	30	66,540	905·0	23·43	905·5	0·06
2	23	43,100	777·1	23·21	785·0	1·01
3	33	36,490	743·5	23·46	743·2	0·04
4	44	32,800	723·3	23·75	713·9	1·32
5	29	25,280	668·4	23·80	658·6	1·49
6	9	16,860	574·4	23·36	576·5	0·36
7	9	9,984	479·2	23·16	485·2	1·24
		Average		23·45		0·79
		Average, taking into account number of individuals in each group				0·80
		Mean deviation (for groups)				1·05

Similarly, in Table XII the "best n" for females was found to have the value 0·323, with a value for k of 24·80, giving the formula $l = 24{\cdot}80\mathrm{W}^{0{\cdot}323}$. It is possible, however, that for the ultimate accurate determination of n to three places of decimals, and of the corresponding values of the length constant, much more extensive series of data than those dealt with here will be required.

But whether n be taken to two places of decimals or to three, it will be seen on inspection that the curve represented by the formula for males and the curve represented by the formula for females have a point of intersection. At this point, and at this point alone, males and females of equal stem-length are of equal weight. The position of the point in question may be ascertained by equating the expressions $23{\cdot}45\mathrm{W}^{0{\cdot}329}$ and $24{\cdot}80\mathrm{W}^{0{\cdot}323}$, or the expressions $23{\cdot}23\mathrm{W}^{0{\cdot}33}$ and $25{\cdot}60\mathrm{W}^{0{\cdot}32}$. In the former case it is found to correspond to a body-weight of 11,660 grm. and a stem-length of 510·5 mm.

Table XII.—Girls re-grouped—Data and Calculations.

Group.	No. in group.	Average body-weight, W.	Average body-length (stem-length), l.	Length constant calculated. $k = W^n/l$. $n = 0\cdot323$.	Body-length calculated. $l = kW^n$. $n = 0\cdot323$. $k = 24\cdot80$.	Difference between body-length calculated and observed.
		grm.	mm.		mm.	per cent.
1	8	62,960	868·2	24·47	880·2	1·86
2	29	52,540	826·3	24·69	830·3	0·48
3	20	42,350	774·5	24·80	774·5	0·00
4	10	27,250	692·2	25·57	671·6	3·67
5	12	15,440	551·5	24·47	559·0	1·34
6	7	6,070	413·1	24·78	413·5	0·10
		Average		24·80		1·16
		Average, taking into account number of individuals in each group				0·98
		Mean deviation (for groups)........				1·86

In the latter case it corresponds to a body-weight of 16,220 grm. and a stem-length of 570·4 mm. It is evident that with the data at present in hand it is not practicable to determine the crossing point of the curves more accurately than this. All that can be said at present is that the data for boys and girls show that a crossing takes place somewhere in the region indicated. This may also be seen by comparing the series of body-weights and body-lengths in Table II with those in Table X. Below the point of crossing males are for any given body-length somewhat heavier than females, the difference becoming more marked as the body-length diminishes. Above the crossing the males are for any given body-length somewhat lighter than females, the difference increasing as the body-length increases.

Distribution of Errors.

I have examined the distribution of errors in the foregoing series of observations by noting the percentage deviation of the observed body-length of each individual boy and girl from the theoretical value of the body-length as calculated by the formula appropriate to the sex. From considerations of space the calculations are not tabulated here, but the results of this examination are set out in Table XIII as compared with the theoretical distribution of errors when the mean deviation is calculated by the method of least squares. The number of observations in which the percentage deviation falls within 0·5, 1, 1·5, 2, 3, and 4 times the mean deviation are

expressed in per cent. of the total number of observations. The actual values are in good agreement with the theoretical requirement.

Table XIII.—Distribution of Errors.

	Theoretical distribution. Percentage of observations.	Distribution for individual boys. Percentage of observations.	Distribution for individual girls. Percentage of observations.
Falling within 0·5 mean deviation ...	38·3	43·5	45·3
" 1·0 " ...	68·3	70·7	77·9
" 1·5 " ...	86·6	85·7	87·2
" 2·0 " ...	95·4	95·9	93·0
" 3·0 " ...	99·7	100·0	100·0
" 4·0 " ...	99·99	100·0	100·0

The mean deviation for boys is 3·378.
The mean deviation for girls is 4·149.

The mean deviation for the girls is about 4·2 per cent. That for boys is less, but the girls cover a much wider range in weight than the boys. Taking the worse figure, namely, 4·2, we may at any rate conclude that if the body-length of an individual deviates by as much as 12 per cent. from the theoretical value calculated from the body-weight by means of the appropriate formula the individual is probably abnormal, and that if the deviation reaches 17 per cent. the individual is certainly abnormal.

Conclusions.

1. Throughout the period of growth from birth to adult age the relationship between the body-weight and body-length (stem-length) in Man conforms to the formula $l = kW^n$.

2. In the male the value of n (to two places of decimals) is 0·33, in the female it is 0·32.

3. The value of the length constant k as determined for grouped individuals is 23·23 for males and 25·60 for females. For the individual boy its average value is 23·33, and for the individual girl 25·58.

4. If the body-length of an individual differ by as much as 17 per cent. from the value calculated by means of the appropriate formula the individual is certainly abnormal; if it differs by 12 per cent. it is probably abnormal.

The relationship between height and weight, and between height and length, is at present under investigation.

Note on an Orderly Dissimilarity in Inheritance from Different Parts of a Plant.

By W. Bateson, F.R.S., and Caroline Pellew.

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In a recent paper* we described the genetic behaviour of the peculiar, wild-looking, "rogue" peas which appear as the offspring of the cultivated types. In several respects the phenomena are as yet without parallel. The genetic constitution of the F_1 plants raised by crossing types with rogues was especially remarkable. These plants, as young seedlings, are intermediate between types and rogues, but, with rare exceptions, as they mature they become normal rogues and behave genetically exactly like pure-bred rogues, producing only rogues as offspring. We conjectured that a segregation of factors takes place in the soma, such that the type elements are left behind in the base of the F_1 plant and are thus excluded from the germ lineage.

The observations here described, though very imperfect, are entirely consistent with the facts related above and with the interpretation offered. As a season must elapse before the evidence can be materially increased, we venture to make this preliminary record.

In the paper referred to we described certain intermediate forms found in Sutton's "Early Giant" (a strain of Gradus). The offspring of these plants shows them to be commonly of two classes: those which throw predominantly rogues and some types, and those which throw predominantly types and a few rogues. The characters of such intermediate plants often change progressively with growth in the direction of the rogue form, the lower parts being more type-like, the upper parts more rogue-like. This fact, taken together with the observation that it is only the lowest leaves of the F_1 plants which show any influence of the type-parent, suggested that when the offspring consists of a mixture of types and rogues, the types may be derived from the lower pods and the rogues from the upper pods. To test this possibility we this year saved the upper and lower pods separately from many plants. The bulk will be sown next spring, but, though the results of autumn sowing are unsatisfactory inasmuch as the plants cannot reach maturity, we have made a preliminary trial with a small quantity of seed. Three families came up which contain the requisite mixture of forms. Several plants were unfortunately damaged by Noctuid larvæ and had to be reckoned as doubtful, but the general result of the experiment is quite clear.

* 'Journ. Genetics,' vol. 5, p. 13 (1915).

	Types.	Dubious.	Rogues.
Family 1.			
Pods at 9th to 11th nodes	2	9	3
Pods at 12th to 15th nodes	—	—	23
Family 2.			
Main stem—			
9th to 14th nodes	2	5	5
16th to 18th nodes	—	3	7
Branch from 2nd node—			
6th to 9th nodes	3	14	—
10th to 12th nodes	—	—	12

From these six sowings it is evident that the two parents were of the kind which throw a majority of plants lower, that is more rogue-like, than the type, together with a few typical plants which were the produce of the lower pods. This was the case both in the main stem and in a branch from the base.

In the third family we had from the 9th to 13th nodes 15 types, and 11 which were almost certainly types, though not well characterised, while from 14th to 17th nodes we had 2 types, 5 below type, and 4 which were almost certainly true rogues. The parent of this family was therefore of the class which throws a large majority of types and a minority of lower forms, and again the lower forms were among the produce of the upper pods.

We have therefore little hesitation in saying that in these plants a segregation takes place in the soma of the plant, such that the type elements are present especially in the lower parts, just as had been surmised from the structure of the F_1 generation.

In view of this evidence it is probable that rogues which arise directly from typical plants are derived predominantly from the apical pods. Material for testing this suggestion will be sown next spring.

Investigations dealing with the Phenomena of Clot Formations. Part III.—*Further Investigations of the Cholate Gel.*

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(Communicated by Prof. V. H. Blackman, F.R.S. Received November 15, 1915.)

In the last communication it was shown that sodium cholate solutions readily set to a gel when heated to 50° in the presence of a calcium salt, and that gel formation is inhibited by the presence of various substances, the inhibitory action of which runs very nearly parallel with their narcotic action and their power of producing cytolysis or exosmosis from living cells.*

These observations are amplified in the present communication by the investigation of the action of the same series of substances on the disintegration of the gel, and of the antagonistic action of certain salts as regards gel formation.

The Disintegration of the Cholate Gel.

Method of Experiment.—A mixture of 2 c.c. of 4-per-cent. solution of sodium cholate, 0·5 c.c. of N/5 calcium chloride and 1·5 c.c. of water was introduced into a capillary tube of 2 mm. internal diameter bent into the shape of a U. This was then placed in a thermostat at 50°, and heated for ½ hour, after which interval the contents had set to a firm gel. The tube was removed from the thermostat and, when cool, cut into lengths of 25 mm. These contained a 2-per-cent. cholate gel with calcium chloride of the concentration of N/40. Single lengths were then introduced into small Erlenmeyer flasks of about 20 c.c. capacity containing 10 c.c. of solutions of varying strengths of the substances under investigation in N/40 calcium chloride solution. After an interval of 16 hours, the capillary tubes were removed from these solutions, and the erosion of the gel from both ends of the tube was measured. The method employed was analogous to that suggested by Mett for measuring the digestive power of solution of proteoclastic ferments.

The results obtained are recorded in Table I. The gramme-molecular equivalents per litre are calculated from the specific gravities of the substances and the solutions determined in the course of the earlier series of investigations.

* 'Roy. Soc. Proc.,' B, vol. 87, p. 366.

Table I.

Per cent. (vol.).	Mol. per litre.	Erosion, mm.	Per cent. (vol.).	Mol. per litre.	Erosion, mm.	Per cent. (vol.).	Mol. per litre.	Erosion, mm.
Ethyl Alcohol.			**Propyl Alcohol.**			**Isopropyl Alcohol.**		
50·0	8·745	>25	25·0	3·220	<25	25·0	3·340	6
25·0	4·372	9	10·0	1·368	6	15·75	2·505	4
10·0	1·759	4	5·0	0·684	4	12·5	1·670	3
5·0	0·875	2	2·5	0·342	2	6·25	0·835	0
2·5	0·437	0						
Butyl Alcohol (Normal).			**Isobutyl Alcohol.**			**Amyl Alcohol.**		
10·0	1·092	>25	10·0	1·108	20	2·5	0·258	8
7·5	0·819	18	7·5	0·831	6	1·875	0·193	6
5·0	0·546	6	5·0	0·554	4	1·25	0·129	4
2·5	0·273	4	2·5	0·277	0	0·625	0·064	0
1·25	0·136	0						
Secondary Amyl Alcohol.			**Tertiary Amyl Alcohol.**			**Ethylene Glycol.**		
						(By weight).		
5·0	0·450	8	5·0	0·460	5	25·0	4·031	6
3·75	0·337	6	3·75	0·345	3	18·75	3·023	4
2·5	0·225	4	2·5	0·230	0	12·5	2·015	0
1·25	0·112	0						
Propylene Glycol.			**Methyl Ethyl Ketone.**			**Methyl Propyl Ketone.**		
(By weight).								
25·0	3·289	6	25·0	2·88	21	5·0	0·484	8
18·25	2·441	5	18·25	2·16	8	3·75	0·363	6
12·5	1·645	4	12·5	1·44	6	2·5	0·242	4
6·25	0·822	0	6·25	0·72	3	1·25	0·121	0
			3·125	0·36	0			
Acetonitrile.			**Chloral Hydrate.**			**Nitromethane.**		
			(By weight).					
25·0	4·95	19	12·5	0·752	>25	10·0	1·505	8
18·75	3·71	14	6·25	0·376	8	7·5	1·127	6
12·5	2·47	8	3·125	0·188	4	5·0	0·752	4
6·25	1·23	4	2·5	0·150	0	2·5	0·376	2
3·125	0·615	0				1·25	0·188	0
Methyl Carbaminate.			**Ethyl Carbaminate. (Urethane).**			**Propyl Carbaminate.**		
(By weight)			(By weight)			(By weight)		
25·0	3·33	>25	25	2·80	>25	6·25	0·606	8
18·75	2·50	>25	18·75	2·10	>25	4·6875	0·457	6
12·5	1·66	10	12·5	1·40	10	3·125	0·303	4
6·25	0·83	5	6·25	0·701	4	1·5625	0·151	0
3·125	0·43	4	3·125	0·350	0			
2·5	0·33	0						
Chloroform.								
Saturated	0·0594	6						
⅘ ,,	0·048	5						
½ ,,	0·0297	4						
¼ ,,	0·0148	2						
⅛ ,,	0·0074	0						

If the above-named substances are arranged in the order of their capacity for destroying a gel, the most potent being placed first in the list, the following series will be obtained.

Table II.

Substances in decreasing order of gel-destroying capacity.	Critical narcotic concentration, mols per litre (Overton).
Chloroform	0·0012
{ Chloral hydrate	0·02
{ Amyl alcohol	0·023
Secondary amyl alcohol	—
Methyl propyl ketone	0·019
Propyl carbaminate	—
Tertiary amyl alcohol	0·037
Butyl alcohol	0·038
Isobutyl alcohol	0·045
Urethane	0·041
Methyl carbaminate	0·27
Propyl alcohol	0·11
Isopropyl alcohol	0·13
Acetonitrile	0·36
Ethyl alcohol	0·3

The order obtained for the gel-destroying power is not exactly the same as that found for the gel-inhibiting action described in the previous communication. It was there mentioned that two substances, viz., methyl propyl ketone and urethane, had a smaller gel-inhibiting action than they should have, on the supposition that this property and the narcotic action run parallel. These two substances in the above Table occupy almost the correct positions if the assumption is made that gel-destruction and narcosis are due to the same physical properties. One other substance of the urethane series of compounds, viz., methyl carbaminate, has also a relatively greater gel-destroying than gel-inhibiting power, and this is the only one in which any marked deviation between the narcotic and gel-destroying powers is demonstrated in the Table given above; it should, if the parallelism between these properties were complete, follow instead of precede the propyl alcohols. In the Table given in the previous paper showing the relationship between narcosis and gel inhibition it occupies the expected position. The polyhydroxy-alcohols have but little action on the gel, and they also exert small narcotic action.

In spite of the one exception, the parallelism between the gel-destroying and narcotic properties is striking.

The Antagonistic Action of Salts.

In the former paper mention was made of the fact that sodium cholate solutions will set to a gel on the addition of large amounts of sodium chloride in the absence of calcium salts. Attempts were made to investigate the combined action of the two salts, and it was found that sodium chloride, when present in relatively small amounts, instead of accelerating the gel-forming action of calcium salts, exerted a marked inhibitory effect.

In the first series of experiments on this subject attempts were made to measure the clotting time in the presence of calcium salts and varying quantities of sodium and other salts by the method described in the earlier paper. The concentration of calcium chloride chosen was N/40, but the clot-formation was so gradual under the conditions chosen that no accurate results could be obtained. Attempts were therefore made to ascertain the amounts of various salts which were necessary to inhibit entirely clot-formation when the various mixtures were heated in a thermostat at 50°. The effect of the four following salts on the clot formation by calcium chloride was investigated—sodium, potassium, lithium, and magnesium chlorides.

The following series of mixtures were made :—

(*a*)	2 c.c. of 4 per cent.	sodium cholate	sol. + 0·5 c.c.	N/5 $CaCl_2$	sol. + 1·5 c.c.	N	salt sol.
(*b*)	,,	,,	,,	,,	,,	7N/9	,,
(*c*)	,,	,,	,,	,,	,,	5N/9	,,
(*d*)	,,	,,	,,	,,	,,	3N/9	,,

The concentration of the cholate in such mixtures was therefore 2 per cent., and of the calcium chloride N/40. The concentrations of the other salts were in (*a*) 9/24 N, (*b*) 7/24 N, (*c*) 5/24 N, and (*d*) 3/24 N. It was found that after these mixtures had stood for ½ hour in a thermostat at 50°, no clots had formed in (*a*), (*b*), or (*c*), whereas the clot formation in (*d*) was complete. No quantitative difference could be detected between the inhibitory action of the chlorides of lithium, sodium, potassium, or magnesium on the clot formation in the presence of calcium chloride. In all cases a concentration of 5/24 N was sufficient to delay completely the clot formation for ½ hour, whereas the concentration 3/24 N was insufficient.

The solutions of these salts also exert a considerable erosive action on the cholate gel. This statement is illustrated by the following experiments: A gel was formed by heating 2 c.c. of 4 per cent. sodium cholate solution + 0·5 c.c. N/5 calcium chloride solution + 1·5 c.c. water in capillary tubes by the same method as that already described in the experiments on the erosive action of narcotics. Two lengths, each of 25 mm., were then

introduced into Erlenmeyer flasks containing salt solutions of varying strength, and the erosion was measured after 16 hours. The results are given in Table III.

Table III.

	5N/10.	4N/10.	3N/10.	2N/10.	1N/10.
LiCl	50	—	—	—	15
NaCl	50	50	47	28	12
KCl............	50	50	48	28	12
$MgCl_2$.........	50	50	46	35	14

It was found, however, that the addition of calcium salt to the above salt solutions could inhibit their erosive action, and these experiments, described below, offer a striking illustration of the "antagonistic" action of salts.

The same gel in "Mett" tubes was introduced into 20 c.c. of N salt solutions, diluted with an equal volume of calcium chloride solutions of varying strength. One length of 25 mm. was used in each experiment, and the result was measured after 16 hours. The results are given in Table IV.

Table IV.

Solution.	Erosion.
20 c.c. N NaCl + 20 c.c. N/32 $CaCl_2$	25
" " + 20 c.c. N/16 $CaCl_2$	0
20 c.c. N KCl + 20 c.c. N/32 $CaCl_2$	25
" " + 20 c.c. N/16 $CaCl_2$	0
20 c.c. N $MgCl_2$ + 20 c.c. N/32 $CaCl_2$	25
" " + 20 c.c. N/16 $CaCl_2$	8
" " + 20 c.c. N/8 $CaCl_2$	0

In water alone the erosion was 13 mm. after 16 hours, and this was almost entirely stopped by the addition of calcium chloride to the amount of N/64.

It will be seen from the above experiments that the gel is entirely destroyed by solutions of the chlorides of sodium, potassium and magnesium of the concentration N/2. This destructive action is, however, antagonised completely by the presence of calcium chloride in the strength of N/32 in the case of the two first-mentioned salts and in the strength of N/16 in the case of the third; there is, however, but little erosion in the presence of magnesium chloride even when the strength of the calcium chloride is only N/32.

Calcium chloride can also protect the gel against the erosive action of

alkalis, as is demonstrated by the following experiment. "Mett" tubes containing the gel were immersed in the solutions. In N/100 sodium hydroxide solution, the erosion was $>$ 25 mm. By the addition of calcium chloride to the extent of only N/128, the erosion was only very slight.

Discussion of the Results.

In the second paper of this series, attention was called to the striking parallelism between the narcotic and cytolytic action of various organic substances, and their power of inhibiting cholate gel formation. In the present communication it is shown that the above-mentioned biological actions also run parallel with the power of these organic substances to produce gel erosion.

The analogies between biological action of substances and their action on gels has been extended further by the demonstration of the antagonistic action of certain salt solutions. It has been shown, for example, that sodium, magnesium and other chlorides inhibit the gel formation by calcium chloride, and, furthermore, that the gel can be eroded by the said salts, and that the erosive action can be prevented by the addition to the salt solutions of relatively small amounts of calcium chloride. As instances of the antagonistic action of salts the following examples may be quoted. Loeb and Wasteneys* have shown that the *Fundulus* fish will live only a short period in sodium or potassium chloride solutions of the same strength as these salts exist in sea-water. This toxic action of potassium and sodium salts can, however, be antagonised by the addition of small amounts of calcium salts.

Osterhout† has shown that the life of the sea-water plant *Rappia maritima*, which is short in solutions of sodium or magnesium chlorides alone, is very appreciably lengthened by the addition of calcium salts. The same author has investigated other instances of the action of "balanced salt solutions" on plant life and cell permeability, many of which present marked analogies to the antagonistic action of calcium to other salts on the cholate gel described above.

One more instance will suffice to illustrate the biological antagonism of salts. Meltzer and Auer‡ have shown that the injection of magnesium chloride to the amount of 1·7 grm. per kilogramme of body weight into a rabbit produces profound anæsthesia which is rapidly removed by the injection of relatively small amounts of calcium chloride.

The results described in this and the last communication further support

* 'Bio.-Chem. Zeitsch.,' vol. 32, p. 308 (1911).

† 'Botanical Gazette,' vol. 42, p. 127 (1916).

‡ 'Amer. Journ. Physiol.,' vol. 14, p. 361 (1905).

the suggestion already made by the author, that the action of narcotics, cytolytic substances, etc., is due, not to the action on lipoids, as is demanded by the hypotheses of Overton and Hans Meyer, but to their action on some gel-forming substance contained in the magma which holds together the heterogeneous system containing lipoids, proteins, etc., which forms the basis of the cell membrane where such a differentiated structure exists, or even of the whole cytoplasm. A disintegration of the gel structure in the cell by narcotics and cytolytic substances would cause an alteration in the state of aggregation of the heterogeneous system, and an entire derangement of the functions of the cell. If a differentiated cell membrane exists a disaggregation of its gel would alter the permeability. On this hypothesis, furthermore, the biological antagonism of certain salts can be explained, which is not possible with the acceptance of the lipoid theory of Overton and Meyer, unless it is assumed that the salts act on a constituent other than the lipoids. Such an assumption has actually been made by Jacques Loeb to explain the antagonistic action of certain salts in cases which are mentioned above; this investigator suggests that the salts influence the state of aggregation of the proteins.

These instances of biological action of salts, and the analogous facts discovered in the study of the cholate gel, appear to throw some light on the general structure of cell-membranes and cytoplasm. They suggest that these are constituted by a heterogeneous system held together in a magma containing a gel-forming substance possessing many of the physical properties of the cholate gel which have been described in the last two communications dealing with the clot formations from cholate solutions. This hypothesis suggests an explanation not only of the action of organic substances, but also of certain actions of inorganic salts.

Summary.

It is shown that there is a marked similarity between certain vital activities of cells and the behaviour of cholate gel.

1. The erosive action of certain organic substances on the cholate gel runs parallel with their narcotic and cytolytic actions.

2. Gel formation by calcium chloride is inhibited by sodium, magnesium and other chlorides. The same substances can also cause gel erosion, but the erosive action can be antagonised by the addition of relatively small amounts of calcium salts.

3. To explain the parallelism between certain biological actions of organic substances and the antagonistic action of inorganic salts on the one hand and the action of these substances on the cholate gel on the other hand it

is suggested that the cell membrane or cytoplasm is constituted by a heterogeneous system of lipoids, proteins, etc., held together in a magma containing a gel-forming substance with physical properties similar to those of the cholates. On such a hypothesis, the biological action of certain substances can be explained in a manner more satisfactory than is possible by the assumption of the "lipoid" theory of Hans Meyer and Overton.

Gametogenesis and Sex-Determination in the Gall-Fly, Neuroterus lenticularis (Spathegaster baccarum).—Part III.

By L. Doncaster, Sc.D., F.R.S., Fellow of King's College, Cambridge.

(Received January 13, 1916.)

[Plates 6 and 7.]

In two previous papers on the maturation of the eggs and spermatozoa and determination of sex in *Neuroterus lenticularis** I showed (1) that any individual female of the agamic generation produces either male or female offspring, but not both; (2) that the eggs of some agamic females undergo a reduction division at maturation, while those of others do not; (3) that, since males have 10 chromosomes in the germ-cells before maturation, while females have 20, the eggs which undergo reduction produce males, and those which do not, produce females; (4) all eggs of the sexual generation undergo a double maturation division, and are fertilised, giving rise to females of the agamic generation with 20 chromosomes in the ovarian cells. The maturation divisions in the eggs of the sexual generation were described as of a rather peculiar type, and some difficulty was experienced in forming a clear and connected idea of the process.

One important problem remained unsolved—the nature of the difference between the male-producing and female-producing females of the agamic generation. No difference was discernible between the flies of the two types, nor could any difference be found between their chromosome-groups in the ovarian divisions. A tentative suggestion was made that the difference might depend on the existence of two kinds of spermatozoa, one of which might cause the fertilised egg to develop into a male-producing, the other into a female-producing agamic female, and this suggestion seemed to gain

* 'Roy. Soc. Proc.,' B, vol. 82, p. 88 (1910); and B, vol. 83, p. 476 (1911).

some support from the fact that in the spermatogenesis an extra-nuclear body was observed not to divide at the single spermatocyte division, but to pass over into one of the two spermatids.

Further investigation of the matter was interrupted by other work until 1913, but in that year I determined to test the hypothesis mentioned by rearing the offspring of individual sexual females separately, and then finding whether each family contained both male-producing and female-producing individuals. If they did not, that is to say, if the offspring of any one sexual female were all male-producing or all female-producing, the hypothesis that the difference depended on two kinds of spermatozoa would be disproved, for the receptaculum seminis of the fertilised female should contain both sorts of spermatozoa, and therefore both kinds of fertilised eggs should be laid by the one female.* At the same time I determined to test afresh the possibility that the two kinds of agamic females were derived respectively from fertilised and parthenogenetic eggs laid by the sexual females. This seemed very improbable, for in almost every egg of the sexual generation examined for the maturation divisions, a spermatozoon was found, and it was therefore almost certain that all the eggs of the sexual generation are normally fertilised.

For the purpose of these experiments a number of females of the sexual generation were sleeved on oak twigs in May, 1913, one female in each sleeve. Some of the females used had been seen to copulate with males; in other cases two or three males were enclosed in the sleeve with the female so that fertilisation would almost certainly take place, and finally a number of virgin females, from galls kept separately so as to make the access of a male impossible, were also sleeved on other twigs of the same oaks. In several instances virgin females laid eggs in the leaves, but in no case was a gall produced, and the eggs appear either not to have developed at all or to have died at a very early age. It may, therefore, be taken as certain that females of the sexual generation do not reproduce parthenogenetically. Of the fertilised females, about a dozen produced galls on the leaves, some of them in considerable numbers. These galls were allowed to grow until ripe in October, then collected and kept separately through the winter. In February and early March a number of branches of an oak tree were covered with large muslin sleeves to prevent the access of wild flies, and when the galls were about to hatch they were placed in sleeves on the branches which had been covered, and also in some cases on uncovered branches, since the

* If there were two kinds of males, producing different spermatozoa, the result under consideration would still be obtained if each sexual female paired with only one male. This hypothesis is further considered later.

supply of galls was more than enough for the sleeves on the branches which had been covered. About four to eight galls were put in each sleeve, and since some of the parent flies had produced 50–60 galls, in some cases a dozen or more sleeves were used for the galls produced by a single sexual female in the previous summer. In all, over 80 lots of galls were sleeved out. It would probably have been better, in some cases at least, to put only one gall in each sleeve, but as my previous experience had shown that flies when sleeved singly frequently fail to produce any galls, I judged it better to put several galls in each sleeve in order to be more certain of getting a result. As it happened, the season was extraordinarily favourable, and when the leaves appeared those inside the sleeves were crowded with galls. Not quite all were collected, but I hatched and noted the sex of about 9000 flies, as shown in the Table on pp. 186 and 187.*

Inspection of this Table shows at once that the vast majority of flies reared in the sleeves containing galls produced by any one sexual female are of one sex, so that the sexual females A, B, F, H, J, and L had male-producing daughters, while the others had female-producing. In most families, however, there is a small percentage of exceptions, and the question arises, what is the explanation of these? If they occurred exclusively in the sleeves on branches which had not been covered before the galls were sleeved out, it might be assumed that they were produced by very early wild flies which had laid eggs in the buds before the sleeves were put on the tree. They are, however, about as numerous in sleeves on branches which had been covered as on uncovered branches. Further it is to be noticed that the exceptional males in female families are about as numerous as females in male families, and that the number of exceptions varies largely from sleeve to sleeve in the same family. For example, among the flies reared from sexual female C, sleeves 22*a*, 23, 24, 24*a*, 25, 27*a* had no exceptions, with a total of 873 females, while sleeves 21, 22, 27, 28, 29 had a total of 501 females and 33 males, or over 6 per cent. of exceptions. The other families show the same sort of thing. In all, of 80 sleeves (35 male-producing, 45 female-producing), 18 of the male sleeves, containing 1,543 males, and 24 female sleeves, containing 2,561 females, had no exceptions. Thus almost exactly half the sleeves were without exceptions, and these sleeves were evenly divided between the male and female producing, and contained nearly half the total flies reared (4,014 out of 9,574). Frequently, also, the exceptions appeared among the later flies to hatch; this was only definitely recorded in the case

* I take this opportunity of recording my indebtedness to Mr. F. Balfour Browne for his kindness in preserving some of the flies as they hatched, while I was prevented by indisposition from attending to them.

Reference Numbers of Sleeves, above (those which were on branches which had been previously covered *italicised*).
Numbers and Sexes of Flies Hatched in each Sleeve, below.

Sexual female parents.													Totals.
A. Galls sleeved, March 2 ...	*1* 28 ♂	*5* 113 ♂	*7* 71 ♂	—	—	—	—	—	—	—	—	—	212 ♂
B. Galls sleeved, March 2 ...	10 209 ♂ 2 ♀	14 39 ♂ 2 ♀	14A 130 ♂ 28 ♀	15 106 ♂ 1 ♀	15A 58 ♂ —	16 136 ♂ —	18 119 ♂ 2 ♀	18A 42 ♂ —	— — —	— — —	— — —	— — —	839 ♂ 35 ♀
C. Galls sleeved, March 10...	21 16 ♂ 107 ♀	22 2 ♂ 38 ♀	22A — 179 ♀	23 — 143 ♀	24 — 86 ♀	24A — 154 ♀	*25* — 199 ♀	*27* 1 ♂ 22 ♀	*27A* — 112 ♀	*28* 8 ♂ 54 ♀	*29* 7 ♂ 280 ♀	— —	33 ♂ 1374 ♀
D. Galls sleeved, March 10...	30 — 139 ♀	31 — 73 ♀	31A 1 ♂ 202 ♀	*32* — 82 ♀	*33* — 318 ♀	*34* 3 ♂ 118 ♀	*35* 4 ♂ 181 ♀	*37* 6 ♂ 197 ♀	*38* 2 ♂ 103 ♀	*38A* — 66 ♀	*39* 1 ♂ 71 ♀	*39A* 7 ♂ 133 ♀	24 ♂ 1683 ♀
E. Galls sleeved, March 17...	*40* 35 ♀	—	—	—	—	—	—	—	—	—	—	—	35 ♀
F. Galls sleeved, March 11...	41 259 ♂ 1 ♀	42 267 ♂ 4 ♂	42A 186 ♂ —	43 192 ♂ 2 ♀	*44* 70 ♂ —	*45* 79 ♂ 3 ♀	*46* 27 ♂ 8 ♀	*47* 23 ♂ —	— —	— —	— —	— —	1108 ♂ 13 ♀

G. Galls sleeved, March 11…	51 — 54 ♀	51A — 262 ♀	52 7 ♂* 232 ♀	53 — 115 ♀	54 — 77 ♀	54A 9 ♂ 82 ♀	55 — 125 ♀	56 1 ♂ 128 ♀	57 — 18 ♀	58 — 12 ♀	59 23 ♂ 79 ♀	— —	40 ♂ 1184 ♀
H. Galls sleeved, March 11…	60 66 ♂ —	61 51 ♂ —	62 53 ♂ —	63 81 ♂ —	64 92 ♂ 5 ♀	68 109 ♂ —	69 243 ♂ 2 ♀	— —	— —	— —	— —	— —	695 ♂ 7 ♀
I. Galls sleeved, March 17…	71 — 59 ♀	72 1 ♂ 95 ♀	73 — 123 ♀	74 1 ♂ 67 ♀	75 — 25 ♀	76 12 ♂ 87 ♀	77 — 42 ♀	78 — 64 ♀	79 3 ♂ 189 ♀	— —	— —	— —	17 ♂ 750 ♀
J. Galls sleeved, March 17…	80 176 ♂ —	81 30 ♂ —	82 102 ♂ 2 ♀	83 39 ♂ —	84 322 ♂ 5 ♀	85 82 ♂ 1 ♀	86 241 ♂ 2 ♀	87 211 ♂ —	— —	— —	— —	— —	1203 ♂ 10 ♀
K. Galls sleeved, March 17…	110 3 ♂ 113 ♀	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	3 ♂ 113 ♀
L. Galls sleeved, March 17…	125 183 ♂ 18 ♀	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	183 ♂ 18 ♀

Total offspring of females A, B, F, H, J, L, 4235 ♂, 83 ♀ (= 1·92 per cent. ♀).
Total offspring of females C, D, E, G, I, K, 5139 ♀, 117 ♂ (= 2·23 per cent. ♂).
Grand total, 9574, of which 200 (2·09 per cent.) are exceptions.

* The 7 males in No. 52 all hatched after most of the females.

of Series G, sleeve 52, but it certainly occurred in other cases also. These facts strongly suggested that the exceptions were due to the presence of eggs of wild flies, which were remarkably numerous in 1914. It seemed probable that, as the buds on the twigs of necessity pressed against the muslin in some of the sleeves, wild flies might have been able to insert their ovipositors through the meshes of the muslin and lay eggs in such buds. The sleeves used were of muslin of two kinds, one considerably finer than the other. Unfortunately no record was kept of which galls came out of sleeves of the two grades of coarseness, but it seems probable, if the explanation suggested is correct, that the sleeves containing no exceptions were of the finer mesh. I was prevented in the summer of 1914 from repeating the whole experiment, as I wished to do, and an attempt made in 1915 failed because the flies laid no eggs; but, in order to test whether flies can lay through the meshes of muslin, in the spring of 1915 I sleeved a number of twigs, and then put a number of galls in larger sleeves fastened outside the others. Some sleeves were also left on the twigs with no outer sleeve, to see whether galls would be produced in them by wild flies. As this was done before the end of February, two or three weeks before the flies begin to emerge, there is no chance of eggs having been laid in the buds before the sleeves were put on. The result of this experiment was that out of 19 sleeves, each with galls in an outer sleeve enclosing it, three contained galls of the sexual generation in May. In each case the galls were on leaves derived from one bud only; in one sleeve there were 20, in the second 18, in the third 2. From the first two there were reared 17 females in the one case, 15 females and 2 males in the other.

Five twigs were covered with simple sleeves, to see whether galls might be produced in them by wild flies, but none contained any. Although a much smaller proportion of galls were produced inside the sleeves than in the preceding year, this experiment proves conclusively that it is possible for the flies to oviposit through the meshes of the sleeves used, and the smaller proportion is probably due to the fact that the season of 1915 was much less favourable to the flies than that of 1914. Wild galls were extremely scarce at Cambridge as contrasted with their great abundance in 1914, so that if it had not been for the kindness of my friend Mr. E. E. Unwin, who sent me a supply of galls from Reading, I might have not been able to obtain enough to supply sufficient material to complete the cytological work described below. It is probable that the frost and snow of the last days of March, following a mild early spring, killed many of the flies before they had laid. I think it may, therefore, be concluded with certainty that the exceptions recorded in about half the sleeves in the 1914 experiments were due to eggs laid by wild flies.

Maturation of the Fertilised Eggs.

The results of the experiments described make it certain that any individual sexual female produces exclusively, or almost exclusively, male-producing or female-producing agamic offspring. The experiments, therefore, disprove the hypothesis that the two types of agamic female are due to dimorphism of the spermatozoa produced by one male. Two possibilities then remain: the two types of agamic female may be due to two kinds of eggs laid by different sexual females; or, if each sexual female mates with only one male, they may be due to two kinds of males which produce different spermatozoa, as has been suggested by Morgan in the somewhat analogous case of *Phylloxera caryæcaulis.** I have carefully re-examined my preparations of the spermatogenesis in a number of males, and can find no regular differences among them; while not definitely denying their existence, I am unable to find any evidence for them. There is certainly no difference in chromosome number, nor can I find that any chromosome is constantly larger or smaller in some males than in others. It remained, therefore, to discover whether any differences could be found among the eggs laid by different sexual females, and on the analogy of the facts described in my earlier papers on the subject, it seemed possible that such differences might be expected in the maturation divisions. Some indication that such differences might exist was given in the first paper, but as there was then no suspicion that the females were of two types, the eggs of individual females were not preserved separately. It was therefore necessary to collect fresh material. For this purpose, in the spring of 1914, and again in 1915, a number of females were allowed to lay separately, and their eggs preserved in Gilson's acetic alcohol sublimate (absolute alcohol, glacial acetic acid and chloroform, equal volumes; sublimate to saturation) at various ages up to about four or five hours after laying. I have cut sections of these eggs (I wish to record my indebtedness to Mr. D. W. Cutler for valuable help in this part of the work) and have also re-examined all the eggs preserved in 1906 and 1907, on which the description in my previous paper was based. The account that follows is thus derived from a series of over 300 eggs of 15 separate females, in addition to about 200 selected eggs of mixed females. I estimate, however, that fully half of these are in stages either too young or too old to throw any considerable light on the maturation process.

The first suggestion of a difference in the maturation process of eggs of different females was that in some eggs the polar chromosomes, after the

* T. H. Morgan, "The Predetermination of Sex in Phylloxerans and Aphids," 'Journ. Exp. Zool.,' vol. 19, p. 285 (1915).

completion of the maturation divisions, remain separate and distinct, in some cases until the second or third segmentation division of the zygote nucleus, while in other eggs they become clumped together quite early, and cease to be distinguishable in the segmentation divisions. To some extent this difference does appear to be characteristic of the eggs of some females, but it is by no means regular, and I think it cannot be regarded as a really distinguishing feature.

A second difference in the maturation of eggs laid by different females is found at an earlier stage, but it is difficult to say whether it is really significant. In all cases the nucleus before maturation comes to the surface of the egg, when it is small and evenly stained, and is usually, perhaps always, flattened for a time against the edge of the egg.* The nucleus then enlarges, and its subsequent behaviour seems to show differences in the eggs of different females. The differences between the two types will be described briefly, before giving a more detailed account of the maturation processes. In the eggs of some females it becomes top-shaped, or like two cones attached to each other by their bases, the points of the cones being in a line perpendicular to the surface of the egg (Plate 6, Figs. I, 1 and 2; II, 1 and 2; III, 1). As the nucleus assumes this form, the chromatin becomes concentrated chiefly at the inner and outer points, but there is often also a single or double ring of granules round the equator, or common base of the two cones, which give the appearance of chromatin emitted from the nucleus. In the subsequent stages of these eggs, as will be described more fully below, the separation of the chromosomes which will form the egg-nucleus from the inner polar chromosomes occurs almost simultaneously with the division of the outer group of polar chromosomes, with the result that as a rule three quite distinct groups of polar chromosomes are formed (Figs. III, 7; IV, 5 and 6).

In eggs of other females the process seems to be slightly different. The nucleus appears never to assume the top-shaped form, but swells up into a spherical nucleus containing a well marked reticulum, sometimes with larger aggregations of chromatin at the outer side or round its equator (Plate 7, Figs. VII, 1; VIII, 1). In the later stages of these eggs the separation of the innermost group of chromosomes, which sink in to form the egg nucleus, appears to take place considerably before the division of the outer group, and the latter division is apparently often incomplete, so that only two groups of polar chromosomes may be found in the later stages.

It is not difficult to choose series of eggs of individual females (*e.g.*, Nos. III and VII, Plates 6 and 7) in which the differences described are fairly pronounced, but on the other hand eggs of other females show figures

* *Cf.* Part II of this series, 'Roy. Soc. Proc.,' B, vol. 83, Plate 17, fig. 3.

which it is not easy to place in either group with complete confidence. For example, the eggs shown in Series VI, 1–7 (Plate 7), are intermediate in their early stages between the top-shaped and spherical nuclei, and the later stages, though not very clear, might belong to either type. Similarly, while the early stages of the series shown in Figs. I, 1, 2, are clearly of the top-shaped type, the later figures (I, 3 and 4) might well belong to the second type. While, therefore, there are some indications of two distinct types of maturation process in the eggs of different females, the results obtained do not make these differences so certain as to justify any confident conclusion that they correspond with the male-producing and female-producing offspring.

Whether the differences noticed in the eggs laid by different females are connected with the fact that the flies to which they give rise are either male-producing or female-producing may be doubted, but in any case the nature of the nuclear divisions during maturation is so remarkable as to need a somewhat fuller description. After the nucleus has swollen up, whether it be of the "top-shaped" or "spherical" type, its membrane disappears, usually first at the inner pole (Fig. VIII, 1), and from the nuclear reticulum fine strands are drawn out towards the centre of the egg. Some figures suggest that these are at first loops, but others (*e.g.*, Figs. VIII, 1; IX, 1–6) that they are strands of chromatin not connected with each other at their inner ends. Many figures show that these strands are double at their bases (IV, 3; VII, 4, 5, 6; IX, 1, 2, 3, 4), and when, as not infrequently happens, they appear to arise singly from small masses of chromatin (III, 4; IV, 2; VII, 2), it is possible that these masses are formed by coalescence of two threads in consequence of somewhat defective preservation. In good figures it is usually clear that these strands are about 10 in number, and when only a smaller number is visible it is possibly because some are longer than others, and only the long ones are sufficiently clear of the reticulum to be recognised. While these strands are being formed at the inner side of the nucleus similar but shorter rods or loops are formed also at the opposite side, towards the edge of the egg, and the two series are for a time connected by a network, on which the chromatin is sometimes aggregated into deeply-stained masses.

The subsequent behaviour is extremely hard to follow, and is possibly different in the two classes of eggs, the existence of which has been suggested above. What appears to happen is that the reticulum between the inner and outer rods disappears, perhaps by becoming concentrated into small chromatin masses attached to the proximal ends of the strands (Figs. III, 4; IV, 4), and the inner group of strands then separates itself from the outer

and sinks for a short distance into the egg. The two groups so formed may be temporarily connected by strands of chromatin (Figs. X, 1 and 2). A system of spindle-fibres can then be seen running inwards from the inner ends of the strands, and also, but less clearly, outwards from these strands towards the outer group; in at least one case (Fig. II, 4) the inner spindle-fibres can be seen to be 10 in number. At this stage the strands of the inner group lie as a rule fairly accurately parallel with one another, but show a tendency to become clumped, so that they commonly have the appearance of less than ten moderately thick rods (Figs. I, 3; II, 4; III, 5, 6; VI, 7). The outer strands or rods are often much less regularly arranged, and may appear to cross one another diagonally, or still to be connected by cross-threads (I, 3; VI, 5, 7). I have very few examples of this stage in eggs which belong at all certainly to the second ("spherical nucleus") class, and in them the arrangement of the inner group of strands is so much less regular that it suggests a rather different process (VIII, 2; VII, 7), which will be discussed after the eggs with "top-shaped" nuclei have been considered.

In the eggs of the first class ("top-shaped" nuclei), when the inner group of strands has become arranged as a sheaf of rod-shaped chromosomes on a spindle, the outer group may either follow suit and form a similar group of rods, usually, however, less regular (II, 4; III, 5, 6), or, probably more commonly, a division of the inner group takes place before the outer group has formed any very regular arrangement (V, 1, 2), but in these eggs the division of the outer group takes place quite definitely, though it may lag somewhat behind that of the inner group (IV, 5; I, 4). Although it is quite clear that a double division takes place, I am quite unable to describe in detail how it is effected, for I have failed to find any eggs (except, perhaps, that shown in Fig. X, 2) in stages between that represented in Figs. II, 4, and III, 5, and that shown in I, 4, IV, 5, and VI, 1, 2.

In the earlier stages the chromosomes consist of fairly thick rods (presumably ten, though usually aggregated so as to appear fewer), lying lengthwise on a spindle. In the next stage represented these chromosomes have divided into a group of about ten rods, which sink in to form the egg-nucleus, and ten others, which remain as the inner group of polar chromosomes, but the figures give no indication of whether the division is longitudinal or transverse. The arrangement of the chromosomes on the spindle would suggest a transverse division; the existence of ten rather long, narrow rods in both the egg-nucleus and inner polar group suggests that the division is longitudinal, and this is confirmed by the longitudinal split which is sometimes visible (Fig. X, 1), corresponding with the apparently double

nature of the original strands from which the chromosomes were formed. Without deciding definitely, therefore, it may probably be concluded that the division is longitudinal, and that each of the rods arranged lengthwise on the spindle splits longitudinally, and one half slips over the other and passes to the inner pole, leaving the other nearly stationary. The form of some of the chromosomes represented in Fig. X, 2, might correspond with a division of this sort. That such a division is very unlike what is found in other organisms is obvious, but it appears to be most nearly what the figures observed suggest.

The division of the outer group which follows is perhaps of the same kind, as is indicated by such figures as X, 5, in which some of the separating chromosomes appear to be attached to one another in such a way that the outer end of the inner chromosome is lying parallel and in contact with the inner end of the outer. In other cases the figures give the appearance of a sorting out of the chromosomes of the inner group, rather than of a division (I, 4; X, 4), but the fact that after the two groups have separated some chromosomes may remain connected by long chromatin strands* rather indicates a genuine division. The final result of the division of the outer group of chromosomes in eggs of this class is usually a rather regular group of about 10 chromosomes arranged in a sheaf near the edge of the egg, and a less regular group at a somewhat deeper level (Figs. IV, 5, 6; III, 7).

The above description applies to eggs of females whose eggs in their earlier stages have "top-shaped" nuclei, and my few good figures of eggs of the "spherical nucleus" class make it doubtful whether the divisions are quite similar. The only really satisfactory figure of the stage immediately preceding the division of the inner group of chromosomes is shown in Fig. VIII, 2, and it will be seen that in this case the inner group, instead of consisting of a regular sheaf of rod-like chromosomes (*cf.* Figs. II, 4; III, 5) is made up of a rather irregular assemblage of rods and strands. It is possible that this appearance is due to the chromosomes being actually in division, and in process of separation from one another, for the number is clearly more than 10, though the irregular arrangement makes an exact count impossible. A rather later stage is shown in VII, 7, in which eight or nine chromosomes are clearly seen sinking in to form the egg-nucleus, while those of the outer half of the inner group have loop-like connections with one another. In both figures the chromosomes of the outer group show no clear sign of division at this stage, though traces of spindle fibres may be seen running outwards towards the edge of the egg. Fig. VII, 8, *a*, *b*,

* *Cf.* Part I, Plate 2, fig. 32, 'Roy. Soc. Proc.,' B, vol. 82.

shows the polar chromosomes of an egg of this series after the maturation divisions are completed; there is a compact mass derived from the outer half of the inner group (on the right of VII, 8, *a*, with three pieces extending into the section 8, *b*), while the halves of the outer group are intermingled in an irregular mass extending through the two sections. As in the case of the earlier stages, however, various intermediate conditions occur between such figures as VII, 8, and III, 7, and it is possible that the two kinds of eggs described are rather the extremes of a continuous series than sharply distinct types which might be correlated with a difference in sex-production in the flies to which they give rise.

One other point should be noted. In several figures (*e.g.*, VI, 7; VII, 7) there is a more or less conspicuous lagging chromosome, which is possibly derived from one of the chromatin strands which may connect the inner and outer groups after the first division (Figs. X, 1, 2; VI, 5). I have not been able to find any regularity in its occurrence, but if one chromosome differs from the rest in tending to lag in division, the presence of this chromosome in the inner or outer group might possibly be the differentiating factor which determines whether the egg becomes a male-producing or female-producing individual.

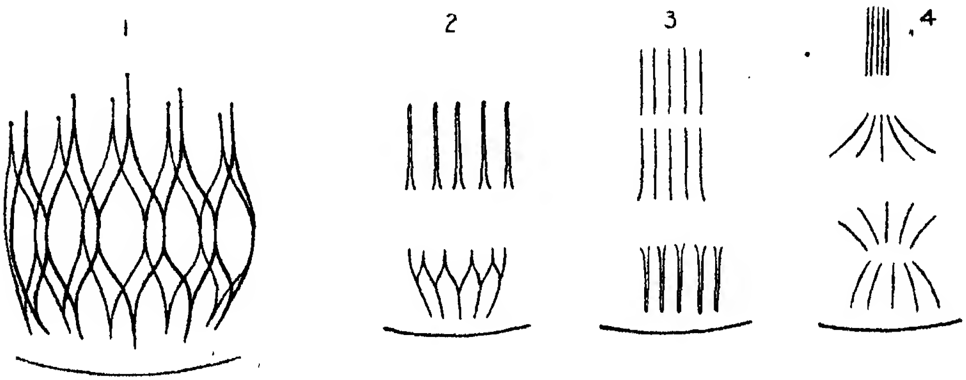
The type of mitotic figure described, which occurs in the maturation division of both the sexual and parthenogenetic eggs of *Neuroterus*, and which is very similar to that described by Henking* in another Cynipid, *Rhodites*, is so different from that commonly found in nearly all other organisms that some further discussion of its peculiarities seems needed. An obvious suggestion is that it is due to defective preservation, and this explanation is supported by the fact that the state of preservation of the eggs seems to differ considerably in eggs fixed together in the same tube. But on the other hand there are strong reasons for believing that this is not the true, or at least not a complete, explanation.

In the first place quite similar figures are found in eggs fixed with Petrunkewitsch's fluid and Gilson's acetic alcohol sublimate, both of which, however, are of similar constitution, but are very different from the fixatives (hot water and Flemming's fluid) employed by Henking. More important reasons for believing that the peculiarities observed are not due to bad fixation are (1) that quite normal and well-preserved segmentation mitoses occur in rather older eggs, and (2), the beautiful clearness and regularity of the fine chromatin strands could hardly be produced by poor fixation. The drawings give only an imperfect representation of the fineness and regularity of the actual figures, owing to the difficulty of rendering them accurately in

* 'Zeit. Wiss. Zool.,' vol. 54, p. 147 (1892).

perspective. Further, although there are considerable differences in detail, in the rather earlier stages especially there is a uniformity about the figures which makes it difficult to believe that the appearances are due to artifact.

I am inclined, therefore, to believe that the figures are at least to a considerable extent genuine representations of what exists in the living egg, and that the maturation divisions of the Cynipidæ exhibit a type of mitosis not hitherto observed in other forms. Its essence appears to be the drawing out of threads from a reticulum; the threads, at least at their bases, are double, and the ends of the longitudinal halves diverge and anastomose with the threads of the network. Gradually the network disappears, apparently becoming absorbed into the drawn out threads, forming an inner and outer group. These two groups then each divide, but, as was said above, there are no clear indications of how this second division is effected; it is probable, however, that the division consists in a separation of the longitudinal halves of the originally double threads. To make this description more intelligible, I give in a text-figure a purely diagrammatic representation of the kind of



DIAGRAMS 1–4.—Representing the kind of mitotic division suggested by the figures observed in the eggs of *Neuroterus*.

1. Threads becoming drawn out on opposite sides of a reticular nucleus.
2. Separation and sinking-in of the inner threads, each of which is longitudinally split.
3. Division (longitudinal ?) of the inner threads, and formation of split chromosomes from the outer group.
4. Completion of the maturation divisions. The innermost group forms the egg-nucleus, the other three the groups of polar chromosomes.

(In diagrams 2, 3, 4, only half of the actual number of chromosomes is represented).

system which the figures suggest, but it will be understood that this representation is imaginary, and is not intended to show at all accurately the structures as they actually exist.

Summary.

In *Neuroterus lenticularis* there are two generations in the year, agamic females appearing in early spring, and sexual females and males in early summer. Previous work had shown that any individual agamic female has only male or only female offspring, and the object of the present work was to discover the nature of the difference between these two classes of agamic females.

Experiment showed that any individual sexual female has grandchildren exclusively or almost exclusively of one sex. The galls produced by sexual females were sleeved, each sleeve containing galls derived from one female parent, and in sleeves of galls derived from six females 4235 males and 83 females were bred, while in sleeves of galls from another six females there were 5139 females and 117 males. About half the sleeves contained no exceptions, and reasons are given for believing that the exceptions were due to eggs of wild flies laid through the meshes of the sleeves.

Two possible cytological causes might account for the fact that some sexual females produce only male-producing offspring, whilst others produce only female-producing. If each fly pairs only once, the difference might depend on the existence of two kinds of males, or it might arise through differences in the maturation-processes of eggs laid by the two classes of sexual female. No cytological differences in the spermatogenesis of different males could be detected. The maturation phenomena of the eggs (about 300) of 15 separate females have been examined, and while they seem to fall into two rather distinct types, the differences are not sufficiently considerable to correlate them with the sex-phenomena with any confidence.

The maturation-processes of the eggs are remarkable, and if, as seems probable, the peculiar figures are not due to methods of preservation, they differ widely from the ordinary type of mitosis. The first division takes place by the drawing out of threads (probably double) on each side of the nucleus; the reticulum becomes absorbed in these threads, which form two groups of parallel chromosomes on a spindle. These chromosomes then divide, probably longitudinally, giving rise to the group which forms the egg-nucleus and three groups of polar chromosomes.

[*Postscript, February* 19, 1916.—While the preceding paper was in the press I received R. W. Hegner's paper "Protoplasmic Differentiation in the Oocytes of Certain Hymenoptera."* He describes phenomena in the parasitic Hymenopteran *Copidosoma*, and less fully in the Cynipid *Andricus* (the latter

* 'Journ. of Morphology,' vol. 26, p. 495 (1915).

nearly allied to *Neuroterus*), which may throw important light on the nature of the peculiar mitotic figures found in the maturation divisions of *Neuroterus* eggs. In the early oocyte, after a compact synizesis (contraction phase) a number of thread-like chromosomes emerge from the spireme, appear to pair end to end, and the pairs then arrange themselves as parallel rods on an asterless mitotic figure. Each of these double chromosomes shows its double nature by a transverse projection in the middle, so that it has the form of a cross with the horizontal arms very short. The whole group of chromosomes then condenses till it makes an evenly stained mass which is more or less oval in shape. This is the last stage seen before the egg is laid, and evidently corresponds with the small evenly-stained nucleus at the edge of the egg found in eggs of *Neuroterus* preserved immediately after deposition. It is extremely like Figs. 1 and 2 of Plate 17 in Part II of this series.*

Further, F. Martin† finds the same phenomena in *Ageniaspis*, followed by a maturation division apparently of the type described in *Neuroterus*.

It seems very probable, therefore, that the "top-shaped" nucleus described in *Neuroterus* is a stage in the disentangling of these chromosomes from the compact condition, and that the separation of the inner group from the outer in the first division corresponds with a separation of the members of the end-to-end pairs from one another, while the second division would be, as suggested above, a longitudinal splitting of single chromosomes. The peculiarities of the first division may be supposed to arise from the fact that the double chromosomes prepare for division some time before the egg is laid, then become closely pressed together into a compact mass, and after the egg is laid separate from one another before they are completely disentangled from the tight mass into which they have been concentrated.]

* 'Roy. Soc. Proc.,' B, vol. 83 (1911).

† 'Zeit. Wiss. Zool.,' vol. 110, p. 419 (1914).

DESCRIPTION OF FIGURES.

The figures are all freehand drawings made with a Zeiss 2·5 mm. immersion lens (N.A. 1·40), and compensating ocular 12. They are not all exactly on the same scale. In many of the figures, especially those representing the earlier stages of the maturation divisions (*e.g.*, Series IX), the difficulty of interpreting and representing the structures in perspective with complete accuracy has proved insuperable, and the figures must therefore be taken as giving as close a rendering as I am able to do of the appearance presented, rather than as drawings which are accurate in every detail. In each case the line on the left of the figure represents the position of the egg-margin. The Roman numerals represent (except in Nos. IX and X) different individual females, so that, for example, Figs. VII, 1–8, represent eight maturation figures from eggs laid by one parent. Series IX and X represent figures at various stages of division from eggs of mixed females; of these, Figs. IX, 1–6, and X, 3 and 5, were preserved in 1907; X, 1, in 1906; X, 4, in 1914, and X, 2, in 1915. All the eggs of Series I–VIII were preserved in 1914 or 1915.

SERIES I, 1, 2.—"Top-shaped" type of nucleus just before maturation. In I, 2, the stained granules, suggesting emission of chromatin, are seen round the equator.

I, 3.—Early stage of division; since in some cases the chromosomes are lying one directly over another not all are shown.

I, 4.—Later stage of division. The inner group has divided into egg-nucleus and inner polar group; the outer group has not yet divided. The figure is reconstructed from four successive sections.

SERIES II, 1 and 2.—Top-shaped nuclei with double rings of granules.

II, 3.—Prophase with narrow loops or strands becoming drawn out on the inner side.

II, 4.—Unusually regular division-figure. The inner and outer groups are in successive sections, with the exception of the chromosome marked × in the outer group, which is in the same section as the inner group. On the inner side of the inner group 10 spindle-fibres can be counted with some confidence.

II, 5.—Three groups of polar chromosomes after the completion of the maturation divisions. The lowest and that near the edge are the halves of the outer group and are in one section; the upper on the right hand is the inner group containing 10 chromosomes, and is in the next section.

SERIES III, 1, 2, 3.—Prophases; III, 1, has the typical top-shaped form with equatorial granules.

III, 4.—Chromatin strands being drawn out on both inner and outer sides. Owing to superposition and perspective, not quite everything is shown.

III, 5, 6.—Two double division figures, on rather a larger scale than the remainder. In each case the outer group is in one section, the inner in the next.

III, 7.—Completion of maturation division and sperm-head (*Sp.*) swelling up to form male pronucleus. The two outer groups of polar chromosomes (the outermost with about 10) and the inner group are in one section, with the exception of the chromosomes represented separately below, which are in the next. The egg- and sperm-nuclei (marked ♀ and *Sp.*) are both in the next section but one.

SERIES IV, 1 and 2.—*Early* stages seen in face, cut in two sections. In each the upper figure represents the inner, the lower the outer portion. In IV, 2, there are clearly about 10 threads drawn out towards the egg-centre ; in the lower figure the dark spots are the cut ends which are continuous with the threads in the upper figure.

IV, 3.—Inner portion of a similar figure, showing that the threads, at least near their bases, are double.

(IV, 1, 2, 3, should be compared with VII, 2, 3, 4, 5.)

IV, 4.—Two figures from successive sections of a rather later stage in side view, showing chromatin threads drawn out on both inner and outer side. The staining of this slide (IV, 4) is unsatisfactory, and attempts to improve it have proved unsuccessful. The figures are therefore to be taken as sketches.

IV, 5.—Telophase of maturation division. The egg- and sperm-nuclei are in one section, but are represented nearer together than they actually are ; the two inner polar groups and most of the outer are in the next section but one. Some of the outermost polar chromosomes are hidden by the egg-membrane.

IV, 6.—Polar chromosomes after the completion of the maturation divisions from three successive sections. Those of the inner group are clumped.

SERIES V, 1 and 2.—Two rather similar stages, showing complete separation of egg-nucleus chromosomes from inner polar group before the outer group has divided. In V, 1, the figure is reconstructed from two, in V, 2, from three, successive sections, and the chromosomes are seen nearly in face. The later eggs of this series show three groups of polar chromosomes, indicating that the outer group divides.

SERIES VI, 1, 2, 3.—Three prophases somewhat intermediate between the "top-shaped" and "spherical" types.

VI, 4.—Later stage showing threads, double at their bases, drawn out towards the egg-centre and shorter loops towards the outside. The lower figure represents ends of threads seen in the next section ; the upper figure is slightly spread for the sake of perspective.

VI, 5, 6.—Rather later stages : VI, 5, is cut in two sections ; VI, 6, is probably not well preserved, the inner and outer groups are in successive sections.

VI, 7.—Double division-spindle, with a lagging chromosome in the outer group.

SERIES VII, 1.—Spherical nucleus before maturation.

VII, 2.—Two sections of a later stage in face. The upper figure represents the inner, the lower the outer, portion ; in the latter, the dark spots are the cut ends of threads which appear in the upper figure.

VII, 3.—Oblique view of slightly later stage, showing double threads drawn inwards towards egg-centre.

VII, 4.—Similar stage cut in two sections in face. The upper figure is the inner, and the dark dots in the lower are the cut ends of threads which pass into the upper.

VII, 5.—Similar figure, showing double nature of the threads towards their bases. The 10 double threads forming an irregular outer ring are probably chromosomes ; the three smaller pairs inside the ring are much fainter, and are possibly spindle fibres.

VII, 6.—Oblique view of similar stage.

VII, 7.—Mitosis in side view, showing complete separation of the egg-nucleus chromosomes from the inner polar group, while the outer group shows

little trace of division. The innermost group is in one section, the middle group and most of the outer group in the next. The chromosomes of the outermost group not in this section are represented below.

VII, 8.—Polar chromosomes after maturation is completed, in two successive sections. There is a compact inner group of about 10, and an outer group which contains approximately 20 chromosomes.

SERIES VIII, 1.—"Spherical" type of nucleus with early stage of threads being drawn out towards egg-centre.

VIII, 2.—Double maturation division. There appear to be more than 10 chromosomes in the inner group, and they seem to be in process of sorting out rather than of division. The inner group is in one section; the chromosomes of the outer group marked × in the next, and the rest of the outer group in the next to these.

SERIES IX, 1–6.—Six figures of early stages from eggs of various females, preserved with Petrunkewitsch's solution in 1907, and showing strands or loops of chromatin being drawn out towards both the egg-centre and egg-margin from a nuclear reticulum. In IX, 6, the figure is seen obliquely, and is cut in two sections; the upper portion is the outer.

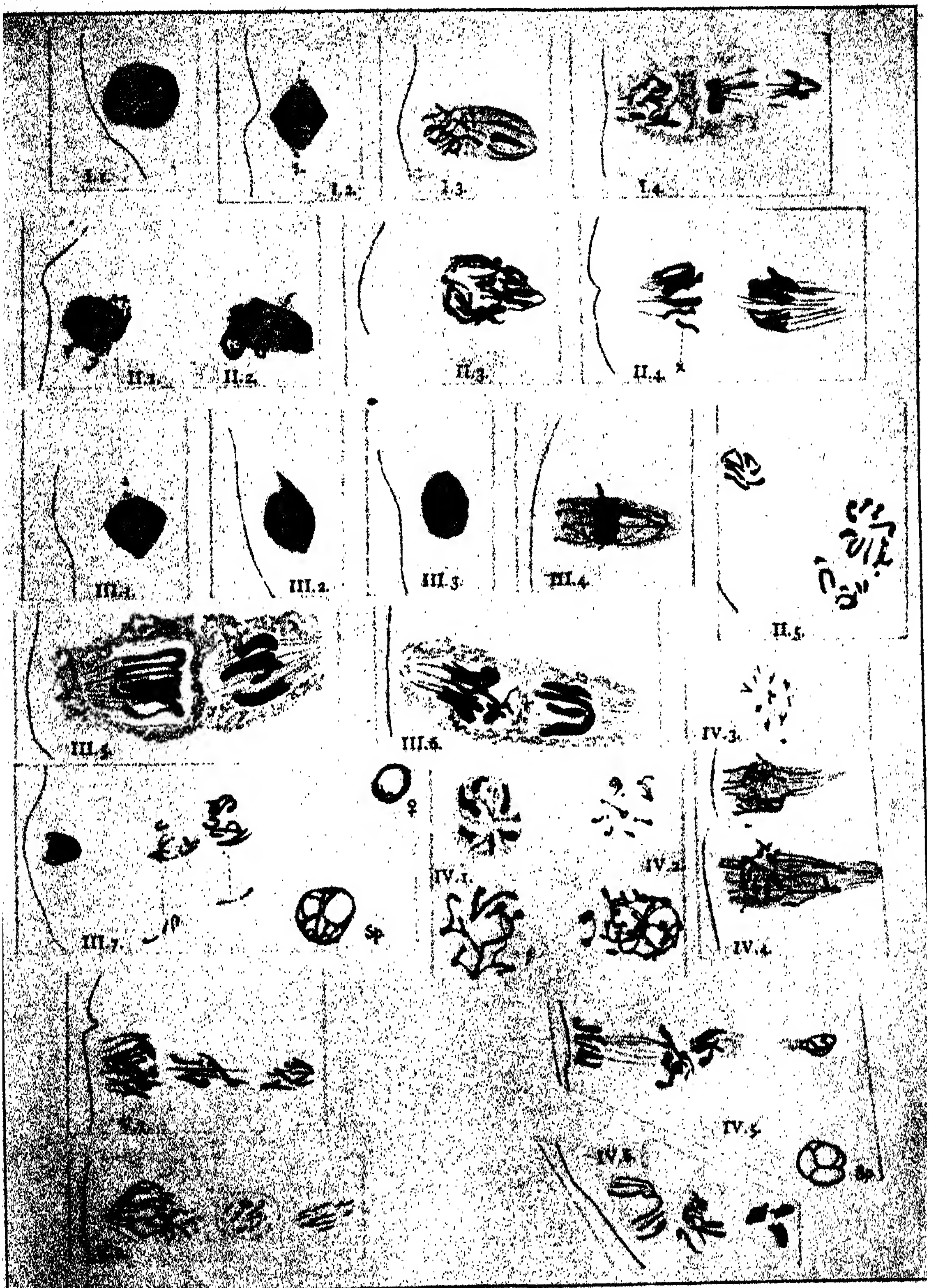
Owing to the difficulty of interpreting and rendering structures seen in perspective, the details of the reticulum as represented are not rigidly accurate, nor is the whole thickness of the nucleus shown in every case.

SERIES X.—Five figures of eggs of various females, later than those shown in Series IX.

X, 1.—Egg preserved in 1906 (Petrunkewitsch's fluid), showing chromatin strands connecting the inner and outer groups. Eight chromosomes, most of them clearly split longitudinally, are visible in the inner group; it is possible that the two long strands represent lagging chromosomes.

X, 2.—Egg preserved in 1915 (Gilson's acetic alcohol sublimate). The chromosomes below those of the inner group on the spindle are in the next section to the remainder. The thick, deeply stained chromosomes of the inner group are probably each two in contact; it is not certain whether the thin faint lines between them are single chromosomes or spindle-fibres.

X, 3–5.—Three stages after the division of the inner group is complete, showing division of the outer group. X, 3 and 5, preserved in 1907; X, 4, in 1914. In X, 4, the outer group and all the chromosomes of the inner, except that marked ×, are in one section; the chromosomes of the egg-nucleus (marked ♀) are two sections removed.


I.1.
I.2.
I.3.
I.4.
II.1.
II.2.
II.3.
II.4.
III.1.
III.2.
III.3.
III.4.
II.5.
III.5.
III.6.
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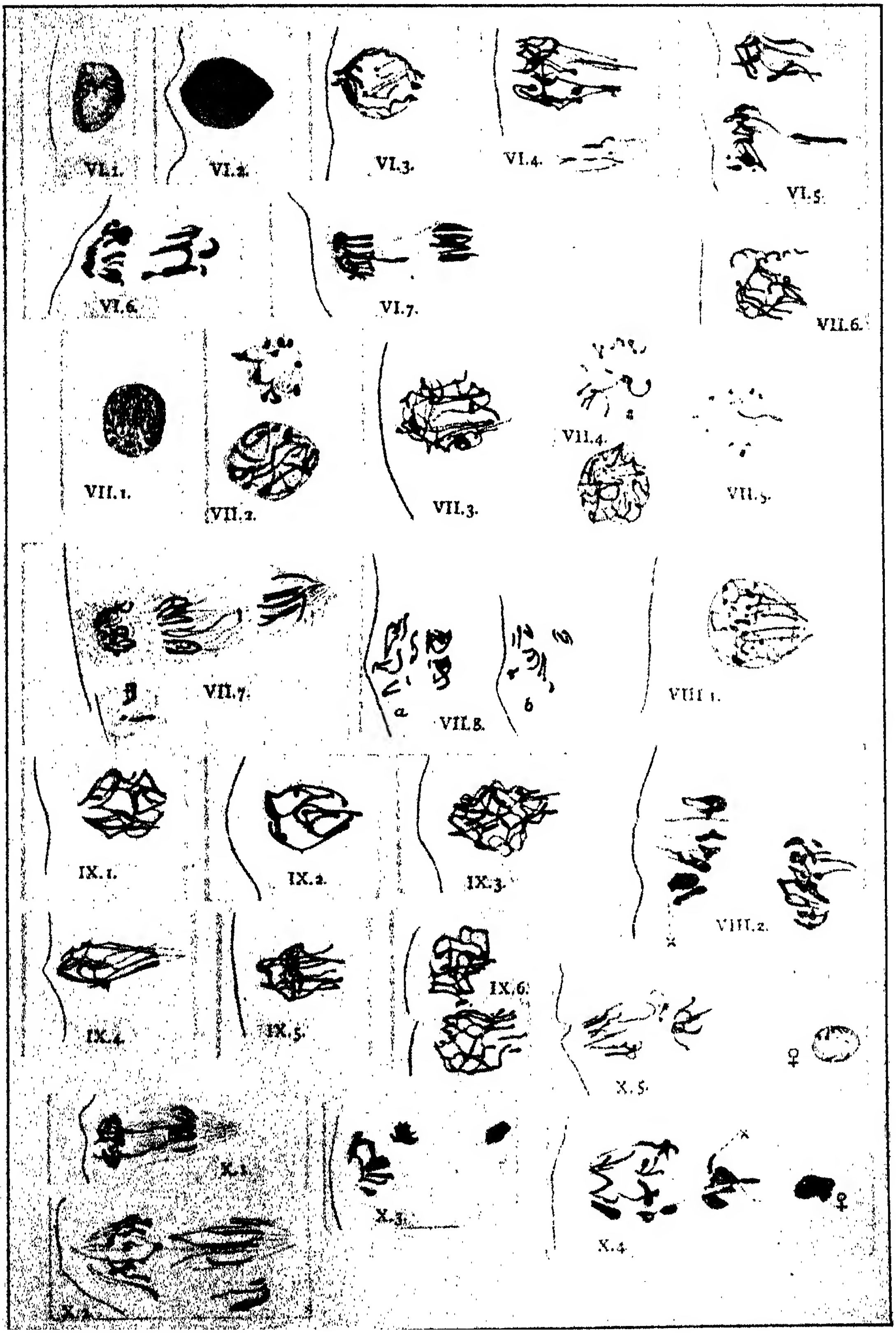
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On the Mechanism of Chemical Temperature Regulation.

By J. M. O'Connor.

(Communicated by J. Barcroft, F.R.S. Received November 18, 1915.)

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Although the conception that, under conditions favouring heat loss, the warm-blooded animal endeavours to maintain its body temperature by increasing the quantity of material oxidised dates back to Crawford and Lavoisier, it received but little attention until examined by Liebermeister (1, 2) in the sixties. Liebermeister's experiments did not carry conviction to his contemporaries, and even when confirmed by Sanders Ezn (3), and by Röhrig and Zuntz (4), were not met with universal acceptance (5). Further experiments by Pflüger (6) and his pupils tended to put the matter beyond doubt, and not alone the existence but the normal activity of such a mechanism was finally established by Herzog Carl Theodor in a careful and prolonged series of observations on a single animal. Voit (8), in whose laboratory these last experiments were conducted, gives an account of the early literature. Since then Rubner (9) especially has devoted attention to this increased combustion, especially studying the manner in which it is influenced by food, growth of fur, and the like.

Though the chemical heat regulation, as Rubner names it, has from this point of view been thoroughly investigated, the mechanism by which its activity is excited has attracted but little attention. Liebermeister (2), it is true, formulated clearly a theory, according to which the increased production of CO_2 in a cold bath is proportional to the extent to which the skin has been cooled below a certain normal point and since his time it has been customary to attribute chemical heat regulation to a temperature reflex from the skin to the muscles (for example, Rubner). The rapid onset of chemical regulation on exposure to cold (Pembrey, 10) makes some such peripheral mechanism probable, but the theory has never been experimentally tested, and is not altogether without opposition. Some would attribute chemical temperature regulation in part (11) or exclusively to a cooling of the brain, a view which has received recent prominent support from the experiments of Barbour (12, 13).

This lack of adequate investigation is perhaps to some extent due to the belief unnecessarily placed on the experiments of Rumpf (14)—for example, Krehl (15)—that in the anæsthetised animal regulation is distorted beyond recognition, thus considerably reducing the possibilities of experiment.

Having found (16) that, in the cat anæsthetised with urethane, the physical heat regulation was in part under the control of the skin temperature, I have endeavoured to find if, under similar conditions, the existence of the mechanism suggested by Liebermeister could be experimentally demonstrated.

The animal, cat or rabbit, was anæsthetised with 1½–2 grm. per kilogramme urethane, and, as a routine, a thermometer or thermocouple introduced deep into the rectum, and two others pushed under the skin at either side. A tracheal cannula was inserted, the thorax opened, and the cannula connected up with an apparatus for artificial respiration, permitting measurement of the O_2 consumption in short periods after the fashion of Regnault and Reiset.

This apparatus (fig. 1) consisted of a mercury pump A, driving and sucking air round in a circuit of rubber tubing under the control of mercury valves, so that, on the down stroke of the pump, the air, having passed through the first valve B, distended the lungs directly connected with the circuit by the tracheal cannula at C, and then forced its way through a wash bottle D, filled with strong potash solution, where the excreted carbon dioxide was absorbed. Beyond this and before the second valve L the circuit communicated through a two-way tap with either of two graduated gas burettes (200 c.c.), F and G, counterpoised by the arrangement H over acidulated water. A small trap M was introduced between the trachea and the circuit to catch any secreted mucus or condensed water, and by means of two tubes O and P across the circuit, furnished with simple taps, the valves could be thrown out of action, equalising the gas pressure throughout the apparatus.

Observations were started as follows: Oxygen was run through the apparatus, and the two-way tap E turned so that one burette was in communication with the air circuit, the tracheal cannula of the animal, on which the preliminary operations had been performed, connected up with the apparatus, and the motor driving the pump put in action. The second burette was filled with O_2 from the gasometer and the quantity read off. After a few minutes the pump was held at the top of its stroke by an assistant, and a stop-watch started. The taps on the bye-passes O and P were opened, throwing all the valves out of action, the burette in connection with the circuit drawn up so as to cause complete collapse of the lungs, and then brought back so that atmospheric pressure prevailed within the apparatus. The two-way tap was turned to bring the other burette into connection, the bye-passes closed, and pump restarted. The whole occupied about 10 seconds, and, normally, did not produce the slightest sign of asphyxia. When the burette was nearly exhausted, the other having been

refilled with oxygen, the same process was again gone through with the modifications of noting the time to the nearest second when the pump was stopped and the reading of the burette. The differences between the two readings of the burette give the oxygen consumed in the time. After each observation the temperatures of the rectum, the skin, and the water of the bath in which the animal was placed, were entered in the protocol.

As owing to the changing temperature of the animal the temperature of

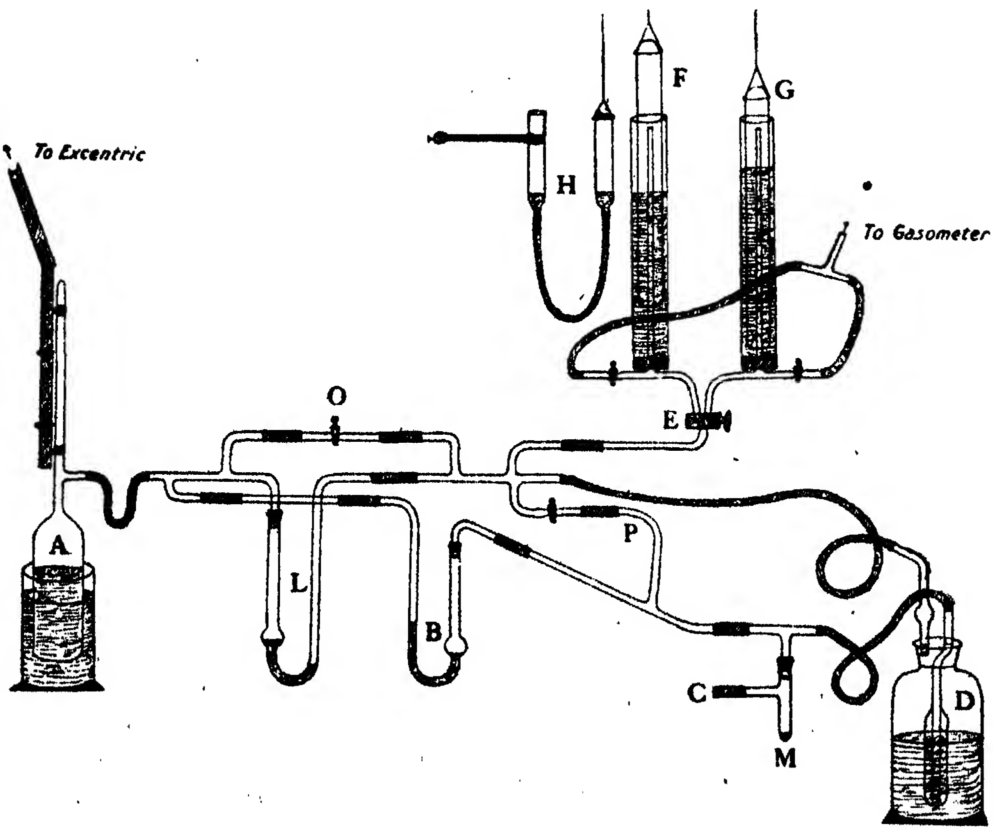

Fig. 1.

the air in the lungs necessarily varied, no effort was made to keep the temperature of the air in the apparatus under control. But as the volume of the apparatus was reduced to a miminum—about 300 c.c. at the middle stand of the burette—the error due to changing temperature or pressure was very small in proportion to quantities of gas (oxygen) consumed. The pump gave a circuit of about 65 c.c. for each downstroke of the pump—thirteen to the minute—which preved an efficient ventilation for rabbits up to 2·5 kgrm. The oxygen in the apparatus was never found below 35 per cent.

and the carbon dioxide never rose above 1 per cent. The airtightness of the apparatus without disconnecting the animal was tested after each experiment.

If in an experiment the beginning of which I have described the animal be left exposed to the room temperature or put in a bath of say 25° C. it shivers violently. This shivering can be seen in the animal without any artificial respiration, but under the conditions of the experiment, provided the ventilation be efficient, is much more pronounced. It is especially marked during the down strokes of the pump. If the temperature of the bath be now gradually raised, the temperatures of the subcutaneous tissue and rectum gradually follow and the shivering becomes noticeably less. The oxygen consumption plotted against the average rectal temperature for these periods gives no constant relation. When, however, the temperatures have risen above a certain point—always less than the normal temperature of the animal—shivering stops completely, and from this point on the oxgyen consumption is within limits directly proportional to the rectal temperature, just as it is in the cold-blooded animal (Schultz, 17). If now this line be extrapolated into the region in which the animal was shivering and the figures obtained be deducted—they are always less—from the observed consumption of oxygen at various rectal temperatures, the difference plotted against the corresponding average skin temperatures gives approximately a straight line. In other words the "extra O_2" consumed during shivering is proportional to the extent to which the subcutaneous temperature has fallen below a fixed point.

The gradient of this extrapolated line varies in different animals. It tends to cut the abscissa at a point between about 19° and 29°, resembling the steep portion of Schultz's curve (see C. S. Martin, 18).

Fig. 2 gives as an example one of many experiments done. Considering the necessary inaccuracies of the observations all accumulating in the final result, the regularity—apart from three outstanding figures—is sufficiently striking.

The lowering of the skin temperature therefore produces an activity of the muscles which is accompanied by, or we may say without prejudice results from, muscular activity. This muscular activity as indicated usually takes the form of a fine incoördinate shiver; but it is not always so. Occasionally in the rabbit the shivering is interrupted by periods of running movements similar to those seen in chloroform narcosis without an obvious alteration in the O_2 consumption. In extreme cold convulsive movements may occur, and frequently there is in addition to the shiver a tonic cramp-like rigidity, easily noticeable on palpation. The fall of skin temperature appears consequently to result in a demand for a certain amount of activity from the muscles, which presumably in the absence of other directive impulses takes the form of a

shiver. The importance of shivering has been especially insisted on by Loewy (19), by Richet (20), and by Johansson (21), who gives a complete sketch

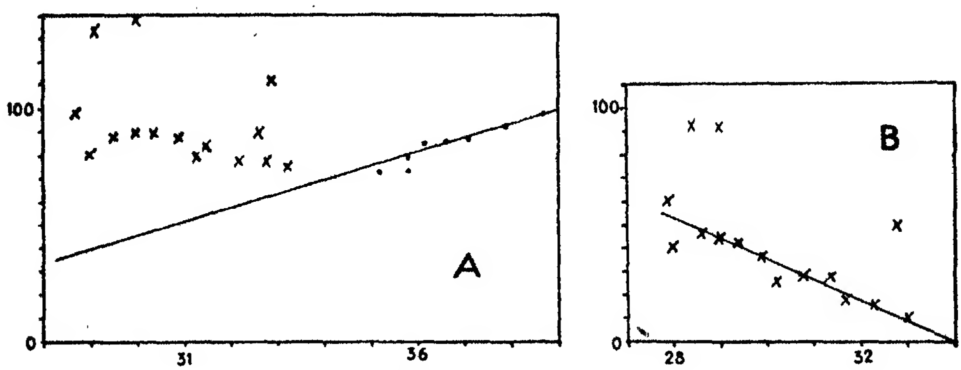

FIG. 2.—Rabbit, 950 grm.

A. Abscissa, rectal temperature. Ordinate, O_2 consumption per 10 minutes. The × indicates observations while shivering. The points indicate observations while not shivering.

B. Abscissa, skin temperature. Ordinate, "extra oxygen."

of the literature of the subject. Rubner (22) believes the significance of shivering to be much exaggerated.

Having found that the anæsthetised animal produced extra O_2 in proportion to the extent to which its skin temperature had fallen below a certain fixed standard, it had to be determined how the animal was aware of the standard to which it should aim. The mechanism was in part revealed by the following experiment. A cat was warmed in the water bath in the usual manner, the O_2 consumption being continuously determined and the heating continued as usual beyond the shivering point. It was then removed from the bath, with the result that the skin cooled rapidly below the shivering point without any muscular activity appearing. The body temperature followed the skin temperature slowly, and when this had fallen below the point at which regulation previously ceased, violent shivering set in, and extra oxygen was consumed in proportion to the extent to which the skin temperature was below that point. The *appearance* of chemical regulation is conditioned by the body temperature being below a fixed point, its *amount* is determined by the extent to which the subcutaneous temperature is below that point. Though among a number of experiments of this type I have met none in contradiction with this view, the results are not always so satisfactory as in that from which fig. 3 is taken. It frequently happens that the animal cooling regulates for a different temperature, generally lower, than that to which it regulated on

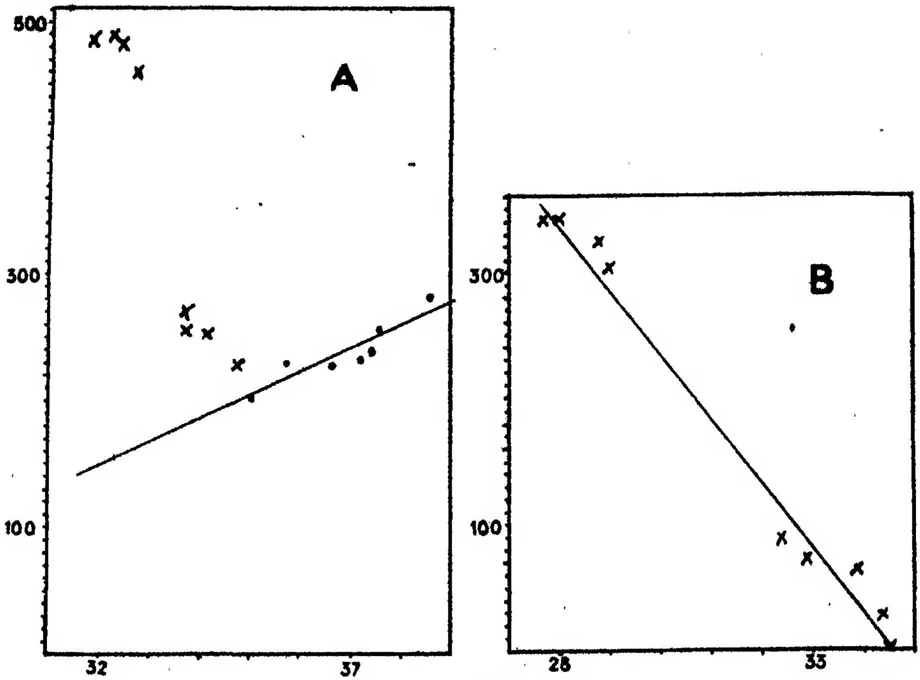

Fig. 3.—Cat, 1950 grm.

A. Abscissa, rectal temperature. Ordinate, O_2 consumption per 10 minutes.

B. Abscissa, skin temperature. Ordinate, extra oxygen per 10 minutes. The four upper points are obtained after having been warmed.

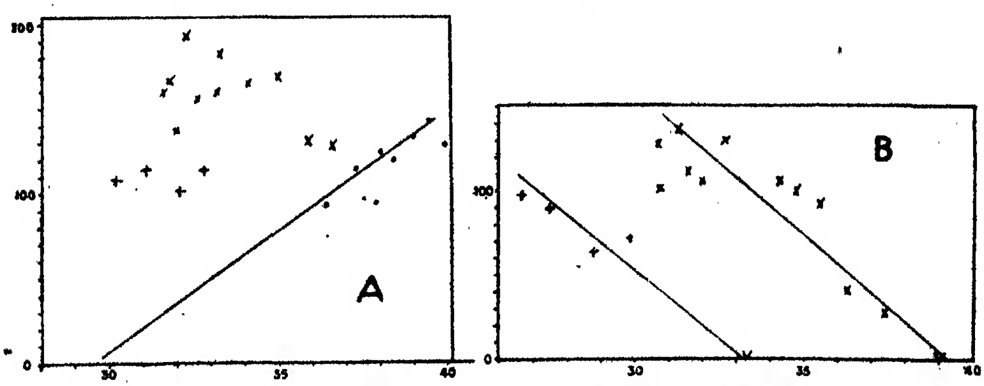

Fig. 4.—Cat, 1250 grm. As fig. 2. × indicates observations when shivering before being warmed. +, shivering after having been warmed.

In both these experiments the O_2 consumption on cooling rose distinctly during the period just before shivering commenced. This is not an invariable occurrence, and is omitted from the graphs.

The correctness of the theory based on these experiments is supported by the fact that it occasionally happens at the beginning of an experiment an animal does not shiver until its rectal temperature has fallen to the point to which it is later found to regulate. This can be seen in the illustrative protocol at the end of the paper.

being warmed, or that the vigour of the regulation was different, *i.e.* the amount of extra oxygen resulting from a given lowering of the skin temperature had altered. As a result the first and second half of the experiment are not in agreement. The first of these cases is illustrated by fig. 4.

This significance of the body temperature having been recognised, an effort was made to determine if this influence could be localised. Probability pointed to the temperature of the brain being of special importance, and in fact it was found possible to completely stop shivering and lower the oxygen consumption by heating the blood going through the carotids (by Kahn's tubes) (23). This is illustrated by the following extract from a protocol:—

Rabbit, 1700 grm.

Time.	O_2 consumption c.c. per 10 min.	Rectal temperature.	Skin temperatures.		Temperature of fauces.	Remarks.
		°	°	°	°	
4.49	187	31·6	30·1	31·1	31·2	Shivering.
4.54	197	31·6	30·0	31·4	34	"
5.3	128	31·8	30·4	31·8	36	Shivering very faint.
5.12	158	32·1	31·2	32·3	36·9	Shivering, which had increased, has stopped.
5.20	98	32·3	30·4	33	38·4	No shivering.
5.28	118	32·1	30·8	32·9	36·7	"
5.32	—	—	—	—	36·1	Shivering recommences.
5.35	128	32	31	32·4	34·8	Shivering.
5.40	144	32	31	32·4	32	"
5.47	165	31·7	31	32·3	31·2	"
5.54	133	31·2	31·2	31·4	31	"

It was hoped by this means that a double series of observations with and without shivering at various rectal temperatures could be obtained, thus avoiding the necessity for obtaining the second figure by extrapolation. A number of such attempts failed on technical grounds. It was found very difficult to get a complete experiment of this type, especially when as was the case the brain temperature was also determined by a thermocouple introduced through the skull. In one experiment I was, however, able to show that shivering ceased at the same brain temperature whether this was reached by locally heating the head blood or warming the body of the animal in the bath. The following shows the essentials:—

Rabbit, 1750 grm.

Time.	Rectal temperature.	Brain temperature.	Remarks.
	°	°	
12.17	29·4	27·7	Shivering.
12.42	28·5	28	"
			Carotid blood warmed.
1.31	29·2	30·6	Shivering.
1.38	29·6	31	No shivering.
2.2	30·2	31·9	No shivering; polypnœa.
2.10	30·8	32	No shivering.
			Carotids no longer warmed?
2.17	31·1	30·4	? Shivering.
2.34	30·8	29·1	Shivering well.
			Bath warmed.
3.8	31·3	29·3	Shivering.
3.20	32·8	30·5	"
3.35	34	31·2	No shivering.
3.56	37·1	32·5	"

On the same grounds of technical disappointments the effort to obtain the important experiment in which the skin was kept above the shivering point while the brain was cooled below it was abandoned. This and similar experiments require essential alteration of technique.

The importance of this experiment lies in the fact that it would effectually clear up any contradictions which might exist between these results and the experiments of Barbour. Barbour and Prince (13) have shown that when the brain is heated there is a fall in the O_2 consumption. This is, of course, explicable by the temperature of the centres rising above the "shivering point." They have, however, also shown that when the temperature of the brain is lowered there is an increase in O_2 consumption. This may constitute a discrepancy. Barbour states that when the brain is cooled there is also a vaso-constriction in the skin. In this way, provided that the temperature of the air is at its usual figure, the skin would become cooler and result in this secondary fashion in an increase in O_2 consumption.

The results obtained point clearly to the theory that while shivering is initiated by a low brain temperature it is quantitatively controlled by the skin temperature. The more accurate localisation of these two significant regions does not come within the scope of this paper. The experiments of Eisenschmid and Schnitzler (22) would point to the tuber cinereum as the essential portion of the brain. With regard to the skin it would be too great a coincidence if the temperature of the subcutaneous region into which the thermometer naturally slips were accurately the governing factor. Liebermeister (2), influenced by the fact that the CO_2 production of fat individuals was less affected by a cold bath than that of the thin, made approximate calculations which led him to believe that the end organs lay beneath the layer of fat on the surface of the muscles. This view is

obviously improbable, and is vitiated by the fact that in his calculations he neglected the latent heat of the fluid subcutaneous fat. This fat, on solidification, would give out large quantities of heat, while, owing to the lack in definition of its melting point, it falls but slowly in temperature. A layer of warmer material is thus retained next the deep surface of the skin in which the end organs for cold lie. Rubner (25) states that at the "cooler places" a fat rich in olein is deposited. I have been unable to find any determination of the latent heat of fusion of fat; beeswax is given as 22.

It may be asked why, if such a complete system of chemical heat regulation exist in the anæsthetised animal, the temperature falls so continuously in an animal exposed even to room temperature. The answer must lie in the fact that though the mechanism is in order the response of the heat-producing organs is not efficient. That this is so is borne out by the fact that the extra O_2 consumption produced by a fall of the skin temperature of 1° C. varies from about 6 to 30 per cent. of the (calculated) basal

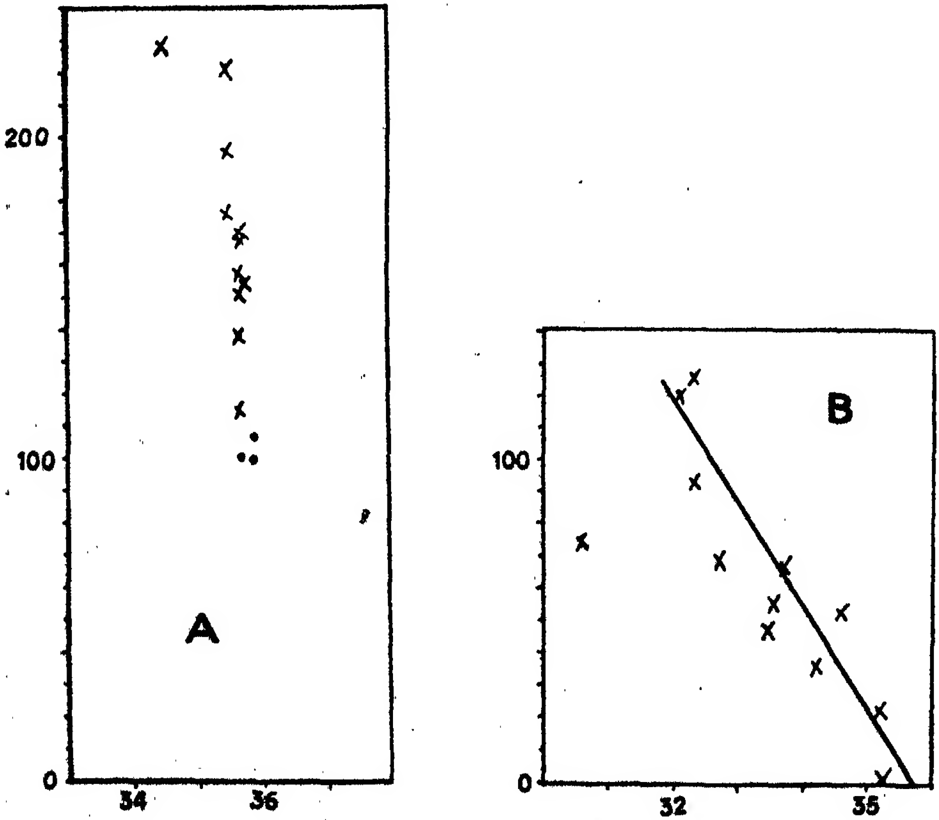

Fig. 5.—Cat, 1650 grm. As fig. 2.

metabolism at 39°. That at times the regulation is capable of maintaining the temperature is shown by the experiment illustrated by fig. 5.

A further point to be considered is the temperature to which the animals chemically regulate. This is always below the normal body temperature (30–39°). I have the impression that the smaller the animal the lower it is, but neither the results nor the conditions are sufficiently uniform to justify a conclusion on this point.

The fact that the anæsthetised animal regulates for a temperature lower than the normal may be due to a direct action on the central nervous system not accessible to further analysis. It may, however, be capable of another explanation. If we conceive the animal unable to regulate and surrounded by certain fixed thermal conditions there will be only one body temperature at which the animal will be in equilibrium, and towards this point it will regulate. If the basal metabolism be lowered the animal will be in equilibrium with the same outside conditions at a lower body temperature and will, according to the hypothesis, now regulate for this lower point. We know that as a matter of fact narcotics acting, for example, on isolated organs (26) do produce a lowering of the metabolism; and the fact that in myxœdema and in starved rabbits (27) a low basal metabolism is associated with a subnormal temperature lends support to the explanation suggested. In fever, too, though the point has scarcely been adequately investigated, the increase of temperature appears to be always accompanied by an increased energy exchange (28). That alterations in the circulation could equally affect the equilibrium point is clear.

The experiments are in consistent agreement with the theory formulated. As an analysis of the normal mechanism of temperature regulation they are not without objection. The periods of observation were very short. It is, of course, true that long periods are more convincing, but, apart from this, Rubner (29) is inclined to question absolutely the value of observations for short periods. Passing the fact that long observations on animals in uniform anæsthesia are scarcely feasible, it must be observed the Rubner objections are arbitrary. It may, of course, be also true that the anæsthesia completely perverts the mechanism in normal activity. The fact that through anæsthesia we are able to reveal aspects of the question otherwise inaccessible must compensate us for this slight suspicion.

Without endeavouring to extend too widely the application of the results, it may be noted that they lend support to Hering's theory of temperature perception, and, if we assume the sensation of cold to be proportional to the amount of oxygen resulting from shivering, do not agree with the Fechner law.

Illustrative Protocol.

March 20, 1915. Male Rabbit, 1610 grm. Temperature 38·8°. 10.20 A.M., 29 c.c., 10 per cent. Urethane *per os.*

Hour.	O_2 consumption.	Time.	O_2 consumption c.c. per 10 min.	Rectal temperature.	Skin temperatures.		Room temperature.	Bath temperature.	Remarks.
		′ ″		°	°	°	°	°	
11.19	—	—	—	36·3	35·7	35·3	9·8	—	No shiver.
11.25	86	5 36	153	36	35·3	35·3	—	—	,,
11.30	88	5 42	154	35·8	35·2	35·1	—	—	,,
11.37	105	7 18	144	35·5	34·5	35	—	—	Slight movements.
11.40	—	—	—	—	—	—	—	—	Shivering ?
11.44	106	7 5	150	35·3	34·8	34·5	—	—	Shivering.
11.50	77	6 1	128	35·1	34·5	34·4	11·8	—	,,
11.56	95	6 17	151	35·0	34·3	34·3	—	—	Shivering. Animal put in bath.
12.3	99	7 44	128	34·2	33·6	35	—	26	Very rigid.
12.11	91	6 58	130	34·5	33	34·7	—	25	Shivering slightly.
12.18	104	7 51	132	34	32	33	—	23	Violent convulsive movements.
12.25	87	6 54	126	33·6	31	33·4	—	27	Shivering.
12.31	78	5 30	142	33·4	31·4	32·3	—	26	,,
12.38	97	7 5	137	33·0	31·4	31·8	—	?	Shivering, alternating with convulsive movements.
12.45	93	6 31	142	33	31·8	31·6	—	28	Shivering.
12.50	78	5 9	151	33	31·8	32	12·6	30	,,
12.57	105	7 9	147	33	32·5	32·5	—	32	,,
1.3	85	5 52	145	33·2	32·9	32·8	—	33	,,
1.8	75	5 41	132	33·4	33·1	33·5	—	34	,,
1.15	71	5 11	137	33·7	34·1	34	—	35	Slight shiver.
1.22	109	8 24	130	34·1	34·8	34·8	—	37	Very slight shiver.
1.27	68	5 18	128	34·5	35	34·9	—	37·5	Just stopped shivering.
1.34	80	6 7	131	35·0	35·6	35·8	—	38	No shiver.
1.39	68	5 8	132	35·4	36·1	36·2	—	38	,,
1.46	95	6 44	141	36	36·7	36·8	—	38	,,
1.50	76	5 11	147	36·3	37·1	37	—	38	,,
1.57	77	5 3	152	36·8	37·6	37·6	—	38	,,

Summary.

1. Anæsthetised cat or rabbits when not shivering consume O_2 in proportion to their body temperature.

2. When shivering more oxygen is consumed than would otherwise be consumed at that body temperature.

3. The onset of shivering is dependent on the brain temperature being below a point more or less fixed in a given animal.

4. The amount of "extra oxygen" consumed during shivering is proportional to the extent to which the average skin temperature is below this point.

5. This point, towards which the animals regulate chemically, varies in different anæsthetised animals between 30° and 39° C.

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Physiological Investigations with Petiole-Pulvinus Preparations of Mimosa pudica.

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(Communicated by Prof. S. H. Vines, F.R.S. Received November 25, 1915.)

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The essential similarity in the responsive reactions of plants and animals which has been demonstrated in my previous works* leads us to expect that the study of the simpler phenomena of irritability in the vegetal organism may help in the elucidation of the more complex physiological reactions of the animal. New methods of investigation found successful in the case of plants may, moreover, prove to be equally applicable to the study of reactions in animal tissues. This anticipation has been fully justified, since it was my previous discovery of a method of inducing variation in the conducting power of vegetable tissues that led me to the discovery of a similar means of control of the excitatory impulse in the animal nerve.†

My investigations on plant-irritability have hitherto been carried out with entire plants. The most suitable plant for these researches is *Mimosa pudica*, which can be obtained in all parts of the world. An impression unfortunately prevails that the excitatory reaction of this plant can be obtained only in summer and under very favourable circumstances, which has militated against its extensive use in physiological experiments, but this misgiving is without any foundation, for I found no difficulty in demonstrating even the most delicate experiments on *Mimosa* before the Meeting of the American Association for the Advancement of Science held last Christmas at Philadelphia. The prevailing outside temperature at the time was considerably below the freezing point. With foresight and care it should not be at all difficult to maintain in a hot-house a large number of these plants in a sensitive condition all the year round.

* Bose, 'Plant Response,' 1906 (Longmans, Green, & Co.); 'Comparative Electro-physiology,' 1907 (Longmans, Green, & Co.); 'Researches on Irritability of Plants,' 1913 (Longmans, Green, & Co.); "An Automatic Method for Investigation of Velocity of Transmission of Excitation in *Mimosa*," 'Phil. Trans.,' B, vol. 204 (1913).

† "The Influence of Homodromous and Heterodromous Electric Currents on Transmission of Excitation in Plant and Animal," 'Roy. Soc. Proc.,' 1915.

In order to remove the drawback connected with the supply of sufficient material, I commenced an investigation to find whether a detached leaf-preparation could be made as effective for the study of irritability as the whole plant. Here we have at the central end of the leaf the pulvinus, which acts as the contractile organ; the conducting strand in the interior of the petiole, on the other hand, is the vehicle for transmission of excitation. The problem to be solved is the rendering of an isolated petiole-and-pulvinus of *Mimosa* as efficient for researches on irritability as the nerve-and-muscle preparation of a frog. On the success of this attempt depended the practical opening out of an extended field of physiological investigation which would be unhampered by any scarcity of experimental material.

In connection with this it is well to note the surprising difference in vegetative growth as exhibited by plants grown in soil and in pots. A pot-specimen of *Mimosa* produces relatively few leaves, but one grown in the open ground is extremely luxuriant. As an instance in point, I may state that for the last five months I have taken from a plant grown in a field about 20 leaves a day for experiment, without making any impression on it. A large box containing soil would be practically as good as the open ground, and the slower rate of growth in a colder climate could be easily made up by planting half a dozen specimens. The protection of the plants from inclemencies of weather can be ensured by means of a glass cover with simple heat-regulation by electric lamps, in place of an expensive greenhouse.

Returning to the question of the employment of an isolated leaf, which I shall designate as a petiole-pulvinus preparation, instead of the entire plant, the first attempts which I made proved unsuccessful. The cut leaf kept in water would sometimes exhibit very feeble response, at other times all signs of excitability appeared to be totally abolished. It was impossible to attempt an investigation on the effect of changing environment on excitability when the normal sensitiveness itself underwent so capricious a change.

These difficulties were ultimately overcome from knowledge derived through systematic investigations on the relative importance of the different parts of the motor apparatus, on the immediate and after-effect of section on the excitability of the leaf, and on the rate of decay of this excitability on isolation from the plant. The experience thus gained enabled me to secure long-continued and uniform sensibility under normal conditions. It was thus possible to study the physiological effects of changing external conditions by observing the responsive variation in the isolated petiole-pulvinus preparation. I propose in this paper to deal with the different aspects of the investigation in the following order:—

1. The effect of wound or section in modification of normal excitability.
2. The change of excitability after immersion in water.
3. Quantitative determination of the rate of decay of excitability in an isolated preparation.
4. Effect of amputation of the upper half of pulvinus.
5. Effect of removal of the lower half.
6. Influence of the weight of leaf on rapidity of responsive fall.
7. The action of chemical agents.
8. Effect of "fatigue" on response.
9. The influence of constant electric current on recovery.
10. The action of light and darkness on excitability.
11. Effect of desiccation and of injury on conducting tissues.

The isolated petiole-pulvinus preparation is made by cutting out a portion of the stem bearing a single lateral leaf. The four diverging sub-petioles may also be cut off. In order to prevent rapid drying, the specimen has to be kept in water. Preparations made in this way often appeared to have lost their sensibility: I was, however, able to trace this loss to two different factors: first, to the physiological depression due to injury caused by section, and, second, to the sudden increase of turgor brought on by excessive absorption of water. I shall now proceed to show that the loss of sensibility is not permanent, but is capable of restoration.

1. *Effect of Wound or Section in Modification of Normal Excitability.*

In connection with the question of effect of injury, it is to be borne in mind that after each excitation the plant becomes temporarily irresponsive and that the excitability is fully restored after the completion of protoplasmic recovery. A cut or a section acts as a very intense stimulus, from the effect of which the recovery is very slow. If the stem be cut very near the leaf, the excitation of the pulvinus is very intense, and the consequent loss of excitability becomes more or less persistent. But if the stem be cut at a greater distance, the transmitted excitation is less intense, and the cut specimen recovers its excitability within a moderate time. I have also succeeded in reducing the excitatory depression by previously benumbing the tissue by physiological means. The isolated specimen can be made still more compact by cutting off the sub-petioles bearing the leaflets; the preparation now consists of a short length of stem of about 2 cm. and an equally short length of primary petiole, the motile pulvinus being at the junction of the two.

For the restoration of sensitiveness, and to meet working conditions, the

lower end of the cut stem is mounted on a T-tube, with funnel-attachment and exit-tube, as shown in fig. 1. The other two cut ends—of the stem and of the petiole—may be covered with moist cloth or may be closed with collodion flexile to prevent rapid evaporation and drying up of the specimen. A slight hydrostatic pressure maintains the specimen in a moderately turgid

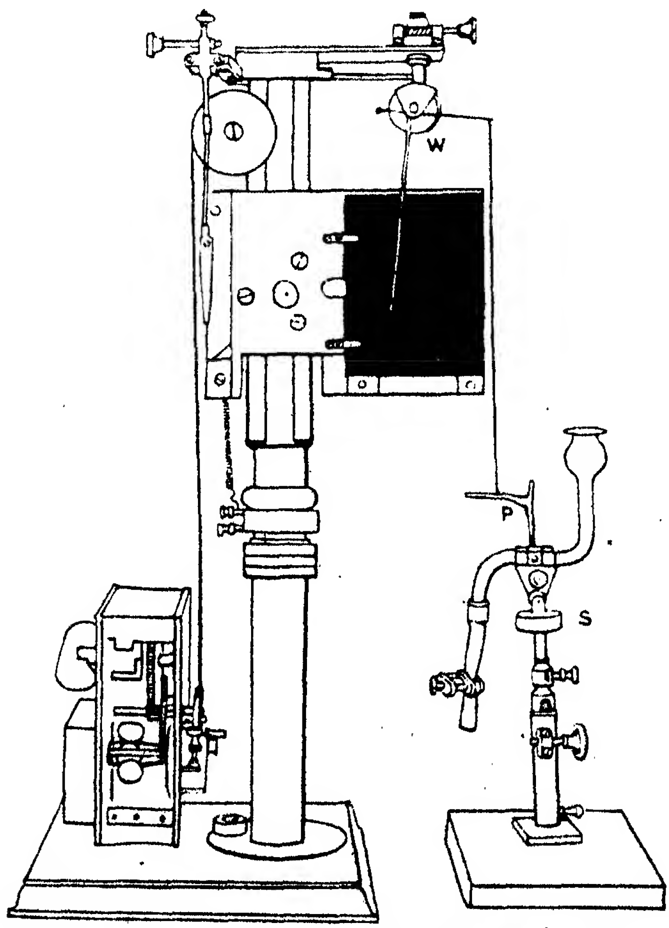


FIG. 1.—The Resonant Recorder, with petiole-pulvinus preparation. (From a photograph.)

condition. A preparation thus made is insensitive at the beginning, but if left undisturbed it slowly recovers its excitability. The history of the depression of excitability after shock of preparation and its gradual restoration is graphically illustrated by a series of records made by the plant (fig. 3).

The petiole-pulvinus preparation thus made offers all facilities for experiment. Owing to its small size it can be easily manipulated; it can be

enclosed in a small chamber and subjected to varying conditions of temperature and to the action of different vapours and gases. Drugs are easily absorbed at the cut end, and a poison and its antidote can be successively applied through the funnel without any disturbance of the continuity of record. In fact, many experiments which would be impossible with the entire plant are quite practicable with the isolated leaf.

The arrangement for taking records of response is seen in fig. 1, which is reproduced from a photograph of the actual apparatus. For recording the response and recovery of the leaf under stimulation, I use my Resonant Recorder fully described in the 'Philosophical Transactions.'* The petiole is attached to one arm of the horizontal lever. The writer, made of fine steel wire with a bent tip, is at right angles to the lever, and is maintained by electromagnetic means in a state of to-and-fro vibration, say, ten times in a second. The record, consisting of a series of dots, is free from errors arising from friction of continuous contact of the writer with the recording surface. The successive dots in the record at definite intervals of a tenth of a second also give the time-relations of the response-curve.

On account of its small size, the petiole-pulvinus preparation offers great facilities for mounting in different ways suitable for special investigations (fig. 2). Ordinarily the cut stem with its lower end enclosed in moist cloth

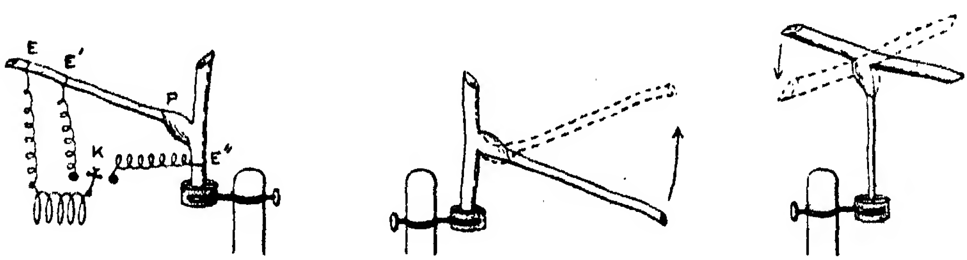


Fig. 2.—Petiole-pulvinus preparation: (*a*) normal position; (*b*) inverted position; (*c*) "wagging" of stem.

is supported below as in (*a*). A very suitable form of stimulus is that of induction shock from a secondary coil, the intensity of which is capable of variation in the usual manner by adjusting the distance between the primary and the secondary coils. The motile pulvinus, P, may be excited directly by applying the exciting electrodes at E and E″. For investigations on velocity of transmission of excitation, stimulus is applied indirectly by means of the electrodes at E and E′. Excitation is now transmitted along the

* *Ibid.*

tract E′P, the conducting power of which will be found appropriately modified under the action of chemical and other agents. In this normal method of mounting, the more excitable lower half of the pulvinus is below; excitatory reaction produces the fall of the petiole, gravity helping the movement. The preparation may, however, be mounted in the inverted position, with the more excitable lower half of the pulvinus facing upwards as in (*b*). The excitatory movement will now be the erection of the petiole, against gravity.

Under natural conditions the stem is fixed, and it is the petiole which moves under excitation. But a very interesting case presents itself when the petiole is fixed and the stem free. Here is presented the unusual spectacle of the plant or the stem "wagging" in response to excitation. In (*c*) the more excitable lower half of the pulvinus is to the left, and under excitation the stem at the left side undergoes a fall, while that on the right is erected. It will be seen how, by having the two lengths of the stem equal, the action of gravity is neutralised.

2. *The Change of Excitability after Immersion in Water.*

The isolated specimen can be kept alive for several days immersed in water. The excitability of the pulvinus, however, undergoes great depression, or even abolition, by the sudden change of turgor brought on by excessive absorption of water. The plant gradually accommodates itself to the changed condition, and the excitability is restored in a staircase manner from zero to a maximum.

In studying the action of a chemical solution on excitability, the solution may be applied through the cut end or directly on the pulvinus. The sudden variation of turgor, due to the liquid, always induces a depression, irrespective of the stimulating or the depressing action of the drug. The difficulty may be eliminated by previous long-continued application of water on the pulvinus and waiting till the attainment of uniform excitability which generally takes place in the course of about three hours. Subsequent application of a chemical solution gives rise to characteristic variation in the response.

3. *Quantitative Determination of the Rate of Decay of Excitability in an Isolated Preparation.*

In order to test the history of the change of excitability resulting from the immediate and after-effect of section, I took an intact plant and fixed the upper half of the stem in a clamp. The response of a given leaf was now taken to the stimulus of an induction shock of 0·1 unit intensity, the unit chosen being that which causes a bare perception of shock in a human being.

The specimen was vigorous and the response obtained was found to be a maximum. The stem bearing the leaf was cut at the moment marked in the record with a cross, and water was applied at the cut end. The effect of section was to cause the maximum fall of the leaf, with subsequent recovery. After this, successive responses to uniform stimuli at intervals of 15 minutes show, in (1) of fig. 3, that a depression of excitability has been induced

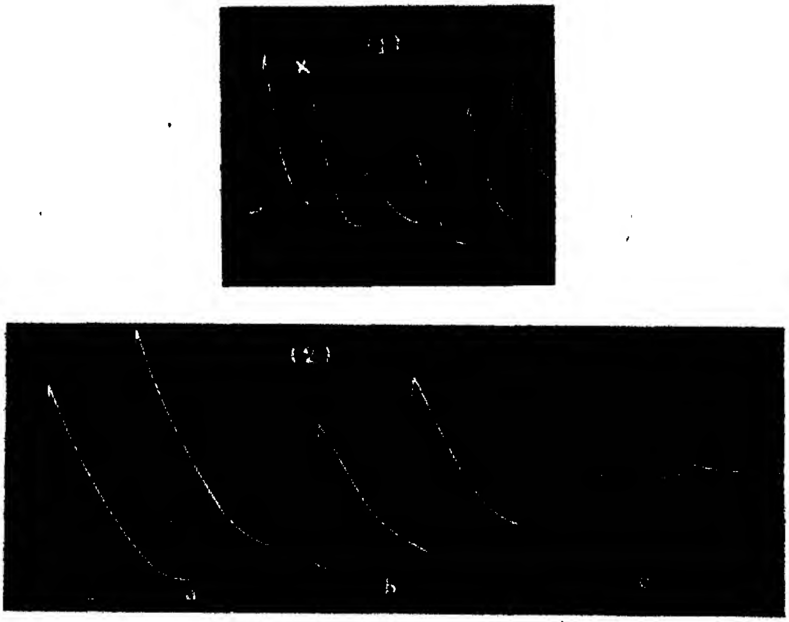


FIG. 3.—Variation of excitability after section. (1) Immediate effect; (2) variation of excitability in a second specimen during 50 hours: (*a*) response 4 hours after section; (*b*) after 24 hours; (*c*) after 49 hours.

owing to the shock caused by section. In course of an hour, however, the excitability had been restored almost to its original value before the section. This was the case with a vigorous specimen, but with less vigorous ones a longer period of about three hours is required for restoration. In certain other cases the response after section exhibits alternate fatigue; that is to say, one response is large and the next feeble, and this alternation goes on for a length of time. The isolated specimen, generally speaking, attains a uniform sensibility after a few hours, which is maintained, with very slight decline under constant external conditions, for about 24 hours. On the third day the fall of excitability is very rapid, and the sensibility declines to zero in about 50 hours after isolation (fig. 3 (2)). We may describe the whole cycle of change as follows: by the shock of operation the isolated preparation is

T 2

rendered insensitive for nearly an hour, the excitability is then gradually restored almost to its normal value before operation. Under constant external conditions, this excitability remains practically constant for about 24 hours, after which depression sets in. The rate of fall of excitability becomes very rapid 40 hours after the operation, being finally abolished after the fiftieth hour. The most important outcome of this inquiry is the demonstration of the possibility of obtaining persistent and uniform sensibility in isolated preparations. On account of this, not only is the difficulty of supply of material entirely removed but a very high degree of accuracy secured for the investigation itself.

4. *Effect of Amputation of Upper Half of Pulvinus.*

The determination of the *rôle* played by different parts of the pulvinus in response and recovery is of much theoretical importance. Our knowledge on this subject is unfortunately very scanty. The generally accepted view is that on excitation "the actual downward curvature of the pulvinus is partly due to a contraction of the walls of the motor cells consequent upon the decrease of turgor, but is accentuated by expansion of the insensitive adaxial half of the pulvinus—which was strongly compressed in the unstimulated condition of the organ—and also by the weight of the leaf."* According to Pfeffer, after excitation of the organ, "the original condition of turgor is gradually reproduced in the lower half of the pulvinus, which expands, raising the leaf and producing compression of the upper half of the pulvinus, which aids in the rapid curvature of the stimulated pulvinus."†

Fig. 4.—Effect of amputation of upper half of pulvinus. (1) Normal response before amputation ; (2) response after amputation (Successive dots at intervals of 0·1 sec.). Apex-time 1·1 sec., in both.

It was held, then, that the rapidity of the fall of leaf under stimulus is materially aided (1) by the expansion of the upper half of the pulvinus, which

* Haberlandt, 'Physiological Plant Anatomy,' 1914, p. 570, English Translation, Macmillan & Co.

† Pfeffer, 'Physiology of Plants,' vol. 2, p. 75, English Translation, Clarendon Press.

is normally in a state of compression, and (2) by the weight of the leaf. So much for theory. The experimental evidence available regarding the relative importance of the upper and lower halves of the pulvinus is not very conclusive. Lindsay attempted to decide the question by his amputation experiments. He showed that when the upper half was removed the leaf carried out the response, but rigor set in when the lower half was amputated. Pfeffer's experiments on the subject, however, contradicted the above results. He found that "after the upper half of the pulvinus was carefully removed, no movement was produced by stimulation, whereas when the lower half is absent a weakened power of movement is retained." Pfeffer, however, adds "since the operation undoubtedly affects the irritability, it is impossible to determine from such experiments the exact part played by the active contraction of the lower half of the pulvinus."*

The cause of uncertainty in this investigation is twofold. First, it arises from the unknown change in irritability consequent on amputation; and, secondly, from absence of any quantitative standard by which the effect of selective amputation of the pulvinus may be measured. As regards the first, I have been able to reduce the depressing action caused by injury to a minimum by benumbing the tissue before operation, through local application of cold, and also allowing the shock-effect to disappear after a rest of several hours. As regards the physiological gauge of efficiency of the motor mechanism, such a measure is afforded by the relation between a definite testing stimulus and the resulting response with its time-relations, which is secured by my Resonant Recorder with the standardised electrical stimulator.

In carrying out this investigation I first took the record of normal response of an intact leaf on a fast moving plate. A second record, with the same stimulus, was taken after the removal of the upper half of the pulvinus, having taken the necessary precautions that have been described. Comparison of the two records (fig. 4) shows that the only difference between them is in the exhibition of slight diminution of excitability due to operation. But, as regards the latent period and the quickness of attaining maximum fall, there is no difference between the two records before and after the amputation of the upper half. The upper part of the pulvinus is thus seen practically to have little influence in hastening the fall.

5. *Effect of Removal of the Lower Half.*

The shock-effect caused by the amputation of the lower half was found to be very great, and it required a long period of rest before the upper half regained its excitability. The excitatory reaction of the upper half is by

* Pfeffer, *ibid.*, p. 75.

contraction, and the response is, therefore, the lifting of the petiole. Thus, in an intact specimen, excitation causes antagonistic reactions of the two halves. But the sensibility of the upper half is very feeble and the rate of its contractile movement, relatively speaking, very slow. The record of the response of the upper half of the pulvinus, seen in fig. 5, was taken with an oscillating recorder, where the successive dots are at intervals of 1 sec.:

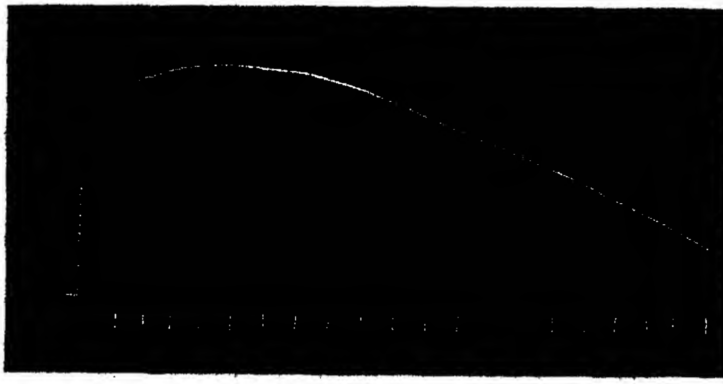


FIG. 5.—Response after amputation of lower half of pulvinus. (Successive dots at intervals of a second; vertical lines mark minutes.) Apex-time, 40 secs.

the magnification employed was about five times greater than in recording the response of the lower half (fig. 4). The intensity of stimulus to evoke response had also to be considerably increased. Taking into account the factors of magnification and the intensity of stimulus for effective response, the lower half I find to be 80 times more sensitive than the upper. Thus, under feeble stimulus the upper half exerts practically no antagonistic reaction. The excitatory response of the upper half is also seen to be very sluggish.

6. *Influence of the Weight of Leaf on Rapidity of Responsive Fall.*

It is obvious that the mechanical moment exerted by the weight of the leaf must help its responsive fall under excitation. But the relative importance of the factors of active contraction of the lower half of the pulvinus and of the weight, in the rate of the responsive down-movement, still remains to be determined. A satisfactory way of solving the problem would lie in the study of the characteristics of response-records taken under three different conditions: (1) When the leaf is helped in its fall by its weight; (2) when the action of the weight is eliminated; and (3) when the fall has to be executed against an equivalent weight. An approximation to these conditions was made in the following manner. We may regard the mechanical

moment to be principally due to the weight of the four sub-petioles applied at the end of the main petiole. In a given case these sub-petioles were cut off, and their weight found to be 0·5 grm. The main petiole was now attached to the right arm of the lever, and three successive records were taken: (1) With no weight attached to the petiole; (2) with 0·5 grm. attached to its end; and (3) with 0·5 grm. attached to the left arm of the lever at an equal distance from the fulcrum. In the first case, the fall due to the excitatory contraction will practically have little weight to help it; in the second case, it will be helped by a weight equivalent to those of the sub-petioles with their attached leaflets; and in the third case, the fall will be opposed by an equivalent weight. We find that in these three cases there is very little difference in the time taken by the leaf to complete the fall (fig. 6).

It has been shown that the presence or absence of the upper half of the

FIG. 6.—Effect of weight on rapidity of fall. N, without action of weight; W, with weight helping; and A, with weight opposing.

pulvinus makes practically no difference in the period of fall; it is now seen that the weight exerts comparatively little effect. We are thus led to conclude that in determining the rapidity of fall, the factors of expansive force of the upper half of the pulvinus and the weight of the leaf are negligible compared to the active force of contraction exerted by the lower half of the pulvinus.

7. *The Action of Chemical Agents.*

In connection with this subject it need hardly be said that the various experiments which I had previously carried out with the intact plant can also

be repeated with the isolated preparation. I will only give here accounts of experiments which are entirely new.

The chemical solution may be applied directly to the pulvinus, or it may be absorbed through the cut end, the absorption being hastened by hydrostatic pressure. The normal record is taken after observing precautions which have already been mentioned in subdivision 2. The reaction of a given chemical agent is demonstrated by the changed character of the record. The effect of the drug is found to depend not merely on its chemical nature, but also on the dose. There is another very important factor—that of the tonic condition of the tissue—which is found to modify the result. The influence of this will be realised from the account of an experiment to be given presently, where an identical agent is shown to produce diametrically opposite effects on two specimens, one of which was in a normal, and the other in a sub-tonic, condition. The experiments described below relate to reactions of specimens in a normal condition.

Hydrogen Peroxide.—This reagent in dilute solution exerts a stimulating action. Normal records were taken after long-continued application of water on the pulvinus. The peroxide, as supplied by Messrs. Parke, Davis & Co., was diluted to 1 per cent., and applied to the pulvinus; this gave rise to an enhancement of response. Re-application of water reduced the amplitude to the old normal value (fig. 7).

Fig. 7.—Stimulating action of hydrogen peroxide.

Barium Chloride.—The action of this agent is very characteristic, inducing great sluggishness in recovery. The preparation had been kept in 1-per-cent. solution of this substance for two hours. After this the first response to a given test-stimulus was taken; the response was only moderate, and the

recovery incomplete. The sluggishness was so great that the next stimulation, represented by a thick dot (fig. 8), was ineffective. Tetanising electric

FIG. 8.—Incomplete recovery under the action of $BaCl_2$ and transient restoration under tetanisation at T.

shock at T, not only brought about response, but removed for the time being the induced sluggishness. This is seen in the next two records, which were taken under the old test-stimulus. There is now an enhanced response and a complete recovery. Beneficial effect of tetanisation disappeared, however, on the cessation of stimulus. This is seen in the next two records, which were taken after two hours. The amplitude of response was not only diminished, but the recovery also was incomplete.

Antagonistic Actions of Alkali and Acid.—Alkali and acid are known to exert antagonistic actions on the spontaneous beat of the heart; dilute solution of NaOH arrests the beat of the heart in systolic contraction, while dilute lactic acid arrests the beat in diastolic expansion. I have found identical antagonistic reactions in the pulsating tissue of *Desmodium gyrans* (the telegraph plant). It is very interesting to find that these agents also exert their characteristic effects on the response of *Mimosa* in a manner which is precisely the same. This is seen illustrated in fig. 9, where the application of NaOH arrested the response in a contracted state; after this, the antagonistic effect of dilute lactic acid is seen first, in its power of restoring the excitability; its continued application, however, causes a second arrest, but this time in a state of relaxed expansion.

$CuSO_4$ Solution.—This agent acts as a poison, causing a gradual diminution of amplitude of response, culminating in actual arrest at death. Certain poisons, again, exhibit another striking symptom at the moment of death, an account of which will be given in a separate paper.

Fig. 9.—Antagonistic action of alkali and acid. Arrest of response in contraction under NaOH (↑), restoration and final arrest in expansion under lactic acid (↑').

8. *Effect of "Fatigue" on Response.*

With *Mimosa*, after each excitation the recovery becomes complete after a resting period of about 15 min. With this interval of rest the successive responses for a given stimulus are equal, and are at their maximum. But when the resting interval is diminished the recovery becomes incomplete, and there is a consequent diminution of amplitude of response. There is thus an increased fatigue with diminished period of rest. This is illustrated in fig. 10, where the first two responses are at intervals of 15 min.; the resting interval was then reduced to 10 min., the response undergoing a

Fig. 10.

Fig. 11.

Fig. 10.—"Fatigue" induced by shortening intervening period of rest.

Fig. 11.—Action of constant current in removal of fatigue by hastening recovery; N, curve of fatigue; C, after passage of current.

marked diminution. Conversely, by increasing the resting interval, first to 12 and then to 15 min., the extent of fatigue was reduced and then abolished.

9. *The Influence of Constant Electric Current on Recovery.*

From the above experiment it would appear that since the incompleteness of recovery induces fatigue, hastening of recovery would remove it. With this idea I tried various methods for quickening the recovery of the excited leaf. The application of a constant electric current was found to have the desired effect. Two electrodes for introduction of current were applied, one on the stem and the other on the petiole, at some distance from the pulvinus. In order to avoid the excitatory effect of sudden application, the applied current should be increased gradually; this was secured by means of a potentiometer slide. In my experiment a current having an intensity of 1·4 micro-ampère was found to be effective. Responses at intervals of 10 min., as we have seen, exhibit marked fatigue. Two responses were recorded on a fast-moving plate, N before, and C after, the application of the current. It will be seen (fig. 11) how the application of current has, by hastening the recovery, enhanced the amplitude of response and brought about a diminution of fatigue. In connection with this, I may state that the tonic condition is, in general, improved as an after-effect of the passage of current. This is seen in some cases by a slight increase in excitability; in others, where the responses had been irregular, the previous passage of a current tends to make the responses more uniform.

10. *The Action of Light and Darkness on Excitability.*

In taking continuous records of responses I was struck by the marked change of excitability exhibited by the intact plant under variation of light. Thus the appearance of a cloud was quickly followed by an induced depression, and its disappearance by an equally quick restoration of excitability. This may be explained on the theory that certain explosive chemical compounds are built up by the photosynthetic processes in green leaves, and that the intensity of response depends on the presence of these compounds. But the building up of a chemical compound must necessarily be a slow process, and it is difficult on the above hypothesis to connect the rapid variation of excitability with the production of a chemical compound, or its cessation, concomitant with changes in the incident light.

In order to find out whether photosynthesis had any effect on excitability, I placed an intact plant in a dark room and obtained from it a long series of responses under uniform test-stimulus. While this was being done the green leaflets were alternately subjected to strong light and to darkness, care being

taken that the pulvinus was shaded all the time. The alternate action of light and darkness on leaflets induced no variation in the uniformity of response. This shows that the observed variation of excitability in *Mimosa* under the alternate action of light and darkness is not attributable to the photosynthetic processes.

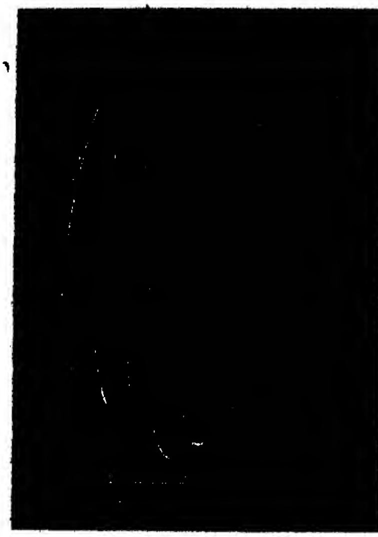


FIG. 12.—Stimulating action of light, and depressing action of darkness.

I next took a petiole-pulvinus preparation from which the sub-petioles bearing the leaflets had been cut off, and placed it in a room illuminated by diffused daylight. The normal responses were taken, the temperature of the room being 30° C. The room was darkened by pulling down the blinds, and records were continued in darkness. The temperature of the room remained unchanged at 30° C. It will be seen from records given in fig. 12 that in darkness there is a great depression of excitability. Blinds were next pulled up, and the records now obtained exhibit the normal excitability under light. The sky had by this time become brighter, and this accounts for the slight enhancement of excitability. This experiment proves conclusively that light has a direct stimulating action on the pulvinus, independent of photosynthesis.

11. *Effect of Desiccation and of Injury on Conducting Tissues.*

I have, in my paper on "Transmission of Excitation in *Mimosa*,"* already referred to, shown that transmission of excitation in the plant is a process fundamentally similar to that taking place in the animal nerve; it has also been shown that the effects of various physical and chemical agents are the same in the conducting tissues of the plant and of the animal.

Effect of Application of Glycerin.—It is known that desiccation, generally speaking, enhances the excitability of the animal nerve. As glycerin, by absorption of water, causes partial desiccation, I tried its effect on conduction of excitation in the petiole of *Mimosa*. Enhancement of conducting power may be exhibited in two ways: first, by an increase of velocity of transmission; and, secondly, by an enhancement of the intensity of the

* 'Phil. Trans.,' B, vol. 204 (1913).

transmitted excitation, which would give rise to an enhanced response of the motile indicator. In fig. 13 are given two records, (1) before and (2) after the

Fig. 13.—Action of glycerin in enhancing the speed and intensity of transmitted excitation.

application of glycerin on a length of petiole through which excitation was being transmitted. The time-records demonstrate conclusively the enhanced rate of transmission after the application of glycerin. The increased intensity of transmitted excitation is also seen in the enhanced amplitude of response.

Action of Injury on Normal Specimens.—A cut stem with entire leaf was taken, and stimulus applied at a distance of 15 mm. from the pulvinus. From the normal record (1) in fig. 14 the velocity of transmission was found to be 18·7 mm. per sec. The end of the petiole beyond the point of

Fig. 14.—Effect of injury, depressing rate of conduction in normal specimen; (1) record before, and (2) after injury. (Intervals, 0·1 sec.).

application of the testing stimulus was now cut off, and record of velocity of transmission taken once more. It will be seen from record (2) that the excessive stimulus caused by injury had induced a depression in the conducting power, the velocity being reduced to 10·7 mm. per sec. Excessive

stimulation of normal specimens is thus seen to depress temporarily the conducting power.

Action of Injury on Sub-tonic Specimens.—I will now describe a very interesting experiment which shows how an identical agent may, on account of difference in the tonic condition of the tissue, give rise to diametrically opposite effects. In demonstrating this, I took a specimen in a sub-tonic condition, in which the conducting power of the tissue was so far below par, that the test-stimulus applied at a distance of 15 mm. failed to be transmitted (fig. 15). The end of the petiole at a distance of 1 cm. beyond

FIG. 15.—Effect of injury in enhancing the conducting power of a sub-normal specimen; (1) Ineffective transmission becoming effective at (2) after section, (3) decline after half an hour, and (4) increased conductivity after a fresh cut.

the point of application of test-stimulus was now cut off. The after-effect of of this injury was found so to enhance the conducting power that stimulus previously arrested was now effectively transmitted, the velocity being 25 mm. per sec. This enhanced conducting power began slowly to decline, and after half an hour the velocity had declined to 4·1 mm. per sec. The end of the petiole was cut once more, and the effect of injury was again found to enhance the conducting power, the velocity of transmission being restored to 25 mm. per sec.

Summary of Results.

On isolation of a petiole-pulvinus preparation, the shock of operation is found to paralyse its sensibility. After suitable mounting the excitability is restored, and remains practically uniform for about 24 hours. After this a depression sets in, the rate of fall of excitability becomes rapid 40 hours after the operation, sensibility being finally abolished after the fiftieth hour.

Experiments carried out on the effect of weight, and the influence of selective amputation of the upper and lower halves of the pulvinus, show that in determining the rapidity of fall of leaf, the assumed factors of the expansive force of the upper half of the pulvinus and the weight of the leaf are negligible compared to the force of active contraction exerted by

the lower half of the pulvinus. The excitability of the lower half is eighty times greater than that of the upper.

Chemical agents induce characteristic changes in excitability. Hydrogen peroxide acts as a stimulant. Barium chloride renders the recovery incomplete; but tetanisation temporarily removes the induced sluggishness. Acids and alkalis induce antagonistic reactions, abolition of excitability with alkali taking place in a contracted, and with acid in an expanded, condition of the pulvinus.

The responses exhibit fatigue when the period of rest is diminished. The passage of constant current is found to remove the fatigue. Response is enhanced on exposure to light, and diminished in darkness. Light is shown to exert a direct stimulating action on the pulvinus, independent of photosynthesis. Application of glycerin on the petiole enhances the velocity of transmission and the intensity of the transmitted excitation.

Injury caused by cut or section of the petiole induces a variation in the conducting power. Two different effects are produced, determined by the tonic condition of the specimen: in normal specimens injury depresses the conducting power, in sub-tonic specimens it enhances it.

The records given are photographic reproductions of the original tracings.

I take this opportunity of acknowledging the special facilities for carrying out these researches that have been afforded me by the Hon. P. C. Lyon, C.S.I., Minister in charge of Education, Government of Bengal.

*The Antiseptic Action of Substances of the Chloramine Group.**

By H. D. Dakin, J. B. Cohen, F.R.S., M. Daufresne, and J. Kenyon.

(Report to the Medical Research Committee.)

(Received February 5, 1916.)

In the course of experiments relating to the employment of antiseptics in the treatment of infected wounds, one of the present writers introduced a modification of the ordinary sodium hypochlorite solution which was found capable of giving useful results when properly employed.† The advantages of this sodium hypochlorite preparation for the treatment of infected wounds compared with some commonly used antiseptics may be referred to the following properties—though powerfully germicidal it does not coagulate blood serum or other protein substances, while at the same time it dissolves necrotic tissue; it is freely soluble and can penetrate to a certain extent, and when properly prepared it is practically non-irritating at 0·5 per cent. concentration.

Out of a very large number of antiseptics that were systematically examined, the hypochlorites seemed to be among the most generally useful. It was, therefore, of interest to study the mode of action of hypochlorites and to endeavour to find related substances which might prove to be of greater practical value.

A number of years ago Raschig showed that when a hypochlorite solution is added to ammonia, the simplest chloramine, NH_2Cl, is formed

$$H_2NH + NaClO = H_2N.Cl + NaOH.$$

This reaction between ammonia and hypochlorite is typical of many similar changes between hypochlorous acid or hypochlorites on the one hand, and more complex amino-compounds on the other. Our knowledge of these changes is chiefly due to the extensive researches of Chattaway. A great variety of organic substances containing (NH) groups react with hypochlorites to give compounds of the chloramine group, *i.e.*, substances containing the : NCl radical.

It appears probable that the germicidal action of hypochlorites is due to chemical reactions of a similar type. It is reasonable to assume that the killing of micro-organisms by antiseptics is due to chemical changes brought

* The work reported in this communication was done partly at the University of Leeds, with the support of the Medical Research Committee, and partly at Hospital 21, Compiègne, France, in laboratories supported by the Rockefeller Institute.

† 'Brit. Med. Journ.,' August 28, October 23, November 27, and December 4, 1915.

about in some of the compounds of the living cell, either by direct action of the antiseptic or by the action of products formed from the antiseptic by combination with substances present in the medium in which the organisms are suspended. Among the chemical substances present in living cells capable of reacting with hypochlorites, the proteins appear likely to play a dominant *rôle*. Under conditions of practical use antiseptics commonly act upon organisms suspended in a medium more or less rich in proteins. Hence hypochlorites might be expected to react on both intra- and extra-cellular proteins.

The action of hypochlorites upon proteins consists, at least in part, in the replacement of the hydrogen of some of the (NH) groups by chlorine, thus forming substances of the chloramine group.* The reaction is somewhat complicated by other changes which will be referred to in more detail later. Now the ability of hypochlorites to attack proteins with the formation of substances containing halogen directly linked to nitrogen appears to be related to their bactericidal action. One piece of indirect evidence may be quoted at once. Free chlorine, bromine, and iodine have not widely differing germicidal power, but when the halogen is converted into hypochlorite or hypobromite, a very marked difference appears. The germicidal action of the hypochlorites, when tested against staphylococci suspended in water, is of a similar order to that of free chlorine, that of hypobromite is only about 1 per cent. of that of free bromine, while a solution of iodine in weak alkali, which may contain unstable hypo-iodite, has a negligible germicidal activity. This feeble germicidal action of hypobromite and hypo-iodite may well be related to their known sluggishness in reacting with proteins and amino-acids compared with the comparative activity of the hypochlorites.† The probability that the formation from proteins of substances containing halogen linked to nitrogen was an intermediate agent in the germicidal action of hypochlorites made it desirable to make a systematic investigation of the germicidal properties of a large number of substances of varied type containing the same (NCl) group. Most of these substances, as will be seen

* Hopkins and Pinkus ('Ber.,' vol. 31, p. 1311, 1898) showed that proteins which had been treated with a large excess of chlorine contained part of the halogen in a loosely combined form capable of liberating iodine from potassium iodide. The view that these substances contained chlorine attached to nitrogen was first put forward by Cross, Bevan, and Briggs ('Journ. Soc. Chem. Ind.,' vol. 27, p. 260, 1908). In a previous communication by one of us (H. D. D.) this paper was unfortunately overlooked.

† Compare Struhetz ('Monatsh.,' vol. 27, p. 601, 1906). Langheld ('Ber.,' vol. 42, p. 2360, 1909) states that gelatine is not attacked by cold sodium hypochlorite. This erroneous conclusion is apparently based on the observation that on mixing the two solutions, unchanged hypochlorite persists, but as a matter of fact the bulk of the hypochlorite readily reacts with the gelatine.

from the Table of Results (p. 243), possess powerful germicidal properties. It is worthy of note that, as shown in the case of the derivatives of acylanilides, marked germicidal properties only appear on the introduction of chlorine attached to nitrogen. The parent substances from which these chloramines are prepared, whether unsubstituted or containing chlorine attached to carbon, show no such action.

The bacteriological tests were made by determining the approximate concentration necessary to sterilise completely one or two drops of a 24-hour-old culture of a vigorous strain of *Staphylococcus aureus* in a total volume of 5 c.c. In a number of cases other suitable organisms, especially *pyocyaneus*, were used as well. Two sets of tests were made, one in which the organisms were suspended in water, the other in which the fluid contained 50 per cent. of horse serum, as it is well known that the presence of serum or other proteins markedly inhibits the action of all known antiseptics. The practical details are noted later.

The substances tested include the following groups:—

Series I.—Chloramine compounds in which the NCl group is separated from a benzene nucleus by the SO_2Na group.
„ II.—Naphthalene derivatives similar to the above.
III.—Other dicyclic derivatives of similar type.
„ IV.—Chloramines in which the NCl group is directly attached to a benzene nucleus.
„ V.—Bromamines.
„ VI.—Products of the action of hypochlorite on proteins, etc.
„ VII.—Certain other compounds for comparison.

A superficial consideration of the results contained in the above Table at once permits certain simple deductions:—

(1) Almost all of the substances examined containing the (NCl) group possess very strong germicidal action.

(2) The presence in the molecule of more than one (NCl) group does not confer any marked increase in germicidal power; compare substances 20, 24, 25, and 26.

(3) The germicidal action of many of these chloramine compounds is molecule for molecule greater than that of sodium hypochlorite.* Thus *p*-toluene sodium sulphochloramide with a molecular weight for the crystallised salt of 261·5 is as active as sodium hypochlorite with a molecular weight of 74.

* Rideal ('Journ. Roy. San. Inst.,' vol. 31, p. 33, 1910) had shown previously that addition of ammonia to hypochlorite with formation of NH_2Cl can lead to an increase over the original germicidal action of the original hypochlorite.

(4) Substitution in the nucleus of aromatic chloramines by Cl, Br, I, CH_3, C_2H_5, or NO_2 groups does not lead to any very great increase in germicidal activity. More commonly there is a moderate diminution.

(5) The chloramine derivatives of naphthalene and other dicyclic compounds of the sulphochloramide type closely resemble the simpler aromatic chloramines in germicidal action.

(6) The few bromamines examined show a slightly lower germicidal action than the corresponding chloramines. But the sodium sulphobromamides are much more active than sodium hypobromite. It is significant that they react much more readily with amino-acids and proteins than does sodium hypobromite.

(7) Derivatives of proteins prepared by the action of sodium hypochlorite and containing (NCl) groups are strongly germicidal. Blood serum inhibits their germicidal action to much the same extent as it does with sodium hypochlorite or the aromatic chloramines.

Mode of Action of Chloramines and Related Substances.

Although it is obvious that nothing more than an incomplete explanation is at present feasible, it is possible to discuss some of the factors regulating the germicidal action of the chloramines.

Chloramines and bromamines are usually more actively germicidal—or at least will kill organisms at a lower molecular concentration—than the corresponding hypochlorite or hypobromite. Hence they cannot be regarded as the biochemical equivalents of the latter substances. It appears as if their germicidal qualities were due to the properties of the complex chloramine or bromamine molecule as a whole, apart from the action of the active halogen in them.

But, on the other hand, we find that chloramines—at any rate, those of the aromatic sodium sulphochloramide type—react with amino-acids, peptones, proteins, and many other substances in very much the same fashion as sodium hypochlorite.

Thus, for example, on adding *p*-toluene sodium sulphochloramide to an aqueous solution of an amino-acid, toluene sulphonamide is precipitated, and the amino-acid is converted into an unstable chloramino-acid, which subsequently decomposes to give an aldehyde and carbon dioxide. In the case of glycine, the reaction may be represented as follows :—

$$CH_3.C_6H_4.SO_2Na : NCl + CH_2(NH_2).COOH = CH_2(NHCl).COONa + CH_3.C_6H_4.SO_2NH_2.$$

$$CH_2(NHCl).COONa + H_2O = H_2C : O + CO_2 + NaCl + NH_3.$$

This change is very similar to the reaction between amino-acids and sodium hypochlorite investigated by Langheld.*

Peptones and proteins react with *p*-toluene sodium sulphochloramide rather more slowly than the amino-acids, judged by the rate of separation of the sparingly soluble toluene sulphonamide. Ammonium salts react rapidly with the sulphochloramide, urea more slowly, while hippuric acid, and probably other acyl-amino-acids, do not react appreciably. These changes in each case resemble the action of hypochlorite. It should be noted, too, that Langheld found that the simplest chloramine, NH_2Cl, reacts with amino-acids like hypochlorite. Judging by these reactions of chloramines, such as toluene sodium sulphochloramide, upon amino-acids, peptones, proteins, ammonium salts, urea, and related substances, it is clear that chloramines can act as chlorinating agents upon important constituents of living cells.

It appears that the aromatic sulphochloramides act as chlorinating agents only when there is opportunity for the chlorine to leave the chloramine and attach itself to nitrogen in a second compound in which the nitrogen atom is united to less acid groups than those in the original substance.

We have found that the aromatic sodium sulphochloramides are readily formed by the addition of sodium hypochlorite to aromatic sulphonamides:

$$R.SO_2NH_2 + NaClO = R.SO_2Na:NCl + H_2O.$$

Since the reactions of the resulting products so closely resemble those of hypochlorites, it might be thought that the above reaction was a reversible one. That this is not the case follows from the following observations:—(*a*) Aqueous solutions of many of the sulphonamides are remarkably stable. Toluene sodium sulphochloramide solutions retain their strength unchanged for months, while if neutral hypochlorite were present, decomposition would be rapid. (*b*) Chloramine solutions do not give the reaction with aniline, indigo, etc., characteristic of hypochlorites.

From the foregoing observations the following tentative conclusions may be drawn:—The fact that many of the constituents of living organisms, including proteins, peptones, amino-acids, urea, and ammonium salts, contain nitrogen in a form capable of attracting the chlorine from chloramines of different kinds, is probably connected with the germicidal action of the latter group of substances. On the other hand, while the chlorinating action of chloramines resembles that of hypochlorites, the germicidal action of the chloramines is often greater than that of an equivalent amount of hypochlorite. This superior germicidal action can be ascribed either to some

* 'Ber.,' vol. 42, p. 2360 (1909).

obscure special action of the chloramine molecule as a whole or possibly to selective chlorination of particular cell constituents.

Practical Use of Chloramines as Antiseptics.—In judging of the suitability of any particular substance for practical use as an antiseptic many factors need consideration. High germicidal activity, when tested against micro-organisms suspended in water is, of course, desirable, but the degree to which this activity is reduced by blood serum, pus, and similar substances is of greater importance, since many antiseptics which act efficiently in watery solutions are practically inert when used on an infected wound. The question of protein coagulation by antiseptics appears to be important, and the fact that hypochlorites and chloramines, unlike many common antiseptics, are not protein coagulants under conditions of practical use is greatly in their favour. Solubility is another important factor. Many of the chloramines, especially those prepared from acylanilides (Section IV in Table), have high germicidal action, but are very sparingly soluble in water. They may be dissolved in vaseline or lanoline, and many of these mixtures were given practical trials. They proved to be of little value, for organisms grew readily under the fatty film. They were deficient in penetrating power, and their use was discontinued. We were therefore led to pay special attention to the chloramines capable of forming soluble sodium salts, and some of these substances appear to be of genuine value.

On the whole, *p*-toluene sodium sulphochloramide seems to be as good as any, and it has already been used successfully for a variety of purposes. The practical applications of the compound have been dealt with elsewhere.* The substance is easily and cheaply made, especially by a method described in the practical part of this paper. It is relatively non-irritating to wounds and can be used in situations where the ordinary antiseptics would prove too irritating or deficient in germicidal action. It may be noted that this compound is readily made from *p*-toluene sulphonic chloride, a cheap bye-product in the manufacture of saccharin. Its manufacture has been undertaken by several British firms, and is sold under the name Chloramine-T.

Reference may be made at this point to a curious compound of hexamethylenetetramine and calcium hypochlorite. It is readily precipitated in the form of fine crystals on adding strong bleaching powder solution to hexamethylenetetramine. The compound has strong antiseptic properties, but is of no practical use, since on keeping in the dry state for a few weeks, it decomposes completely. Substances of this type do not appear to have been previously described. Reference may be made to Delepine's experiments on the action of sodium hypochlorite on hexamethylenetetramine. In

* 'Brit. Med. Journ.,' January 28, 1916.

neutral solution N-dichloropentamethylenetetramine is obtained, while in acetic acid solution trichlorohexahydrotriazine is formed.*

Practical.

Preparation of Substances.—The action of sodium hypochlorite on the sulphonamides was first examined by Raper, Thompson and Cohen.† Later, Chattaway, by a similar reaction, prepared a series of sulphochloramides, details of which can be found without difficulty in the 'Trans. Chem. Soc.' Reference will be made here simply to substances which had not been hitherto obtained by others, or for the preparation of which improved methods have been adopted.

A number of new sulphochloramides were prepared from the corresponding hydrocarbons by successive formation of the sulphonic acid, sulphonic chloride, sulphonamide, and sulphodichloramine. The latter substances were converted into the sodium sulphochloramides by the action of sodium hydroxide according to Chattaway's method.‡

$$R.SO_2.NH_2 + 2HClO = R.SO_2NCl_2 + 2H_2O.$$
$$R.SO_2NCl_2 + 2NaOH = R.SO_2Na : NCl + NaClO + H_2O.$$

The sodium sulphochloramides of the following hydrocarbons had not been previously obtained: *o*-, *m*- and *p*-xylene, ethylbenzene, chlorobenzene,§ dichlorobenzene, bromobenzene, iodobenzene, mesitylene, pseudocumene, dibenzyl. They are all soluble crystalline salts, sparingly soluble in caustic soda solution, and give the general reactions for chloramines. The sodium salts of the sulphochloramides of diphenyl and diphenylmethane, and 2.6 naphthalene sodium sulphochloramide were obtained in solution by decomposing the corresponding dichloramines with hot sodium hydroxide and then neutralising the solution.

We have found that a number of sodium sulphochloramides, including those of benzene and toluene, may be conveniently and economically made by a process which avoids the intermediate preparation of the sulphodichloramine. This method simply consists in dissolving the powdered sulphonamide (1 mol.) in a cold solution of sodium hypochlorite (1.2 mols.), warming and filtering if necessary, and then adding one and a half volumes of saturated brine. The sodium salt of the sulphochloramide crystallises out

* 'Bull. Soc. Chim.' (iv), vol. 9, p. 1025 (1911).

† 'Trans. Chem. Soc.,' vol. 85, p. 371 (1904).

‡ 'Trans. Chem. Soc.,' vol. 87, p. 145 (1905).

§ Since this work was completed, Chattaway has described the three mono-halogen benzene sodium sulphochloramides. 'Trans. Chem. Soc.' vol. 107, p. 1814 (1915).

of solution in about 90 per cent. yield, and is washed with brine and dried in the air.

$$R.SO_2NH_2 + NaClO = R.SO_2Na:NCl + H_2O.$$

The products of the action of sodium hypochlorite on proteins referred to in the Table can hardly be regarded as chemical individuals. They were prepared by adding an excess of hypochlorite, with or without addition of sodium bicarbonate, to a solution of the protein. After a short time the chlorinated product was precipitated either by acidification or by salting out with magnesium or sodium sulphate.

When an excess of sodium hypochlorite is added to blood serum, a certain proportion of the hypochlorite rapidly disappears owing probably to substitution of hydrogen attached to carbon in the various cyclic rings, *e.g.*, tyrosine, tryptophane, histidine. Another portion of the hypochlorite replaces hydrogen attached to nitrogen, and the resulting protein-like substances containing NCl groups when precipitated and washed free from adhering hypochlorite liberate iodine promptly from hydriodic acid. In addition a considerable amount of hydrolysis takes place in the protein-hypochlorite mixture and a certain amount of nitrogen gas is evolved. The formation of aldehydes by the decomposition of the chloramino-acids is also easily noticeable. The reaction between proteins and hypochlorites is a complicated one and will be the subject of further investigation.

The compound of calcium hypochlorite and hexamethylenetetramine previously referred to was prepared as follows:—A clear filtered solution obtained from 50 grm. of good bleaching powder and 200 c.c. of water is added to a solution of 20 grm. of hexamethylenetetramine in 40 c.c. of water. The mixture is at once cooled, when needle-shaped crystals are deposited and fill the liquid. The crystals are filtered off, washed with a little cold water and dried rapidly in vacuo. The yield is 14 grm. The calcium in this compound can be removed by oxalic acid or carbon dioxide, giving a faintly green solution with marked bleaching properties. The substance decomposes rapidly on keeping for two or three weeks.

Analyses of freshly prepared specimens showed that almost all the chlorine was in the hypochlorite form and that the ratio of chlorine to nitrogen was exactly 2 : 4. The proportion of calcium in the undehydrated substance (14·7 per cent.) was always too high for the simple formula

$$(CH_2)_6N_4.Ca(OCl)_2. \qquad (Ca = 14{\cdot}1 \text{ per cent.})$$

The ability of hexamethylenetetramine to form complex salts is well known, and this may account for the excess of calcium.

Determination of Germicidal Action.—The antiseptic action of the substances referred to in the Table was characterised by determining the minimal concentration of a solution capable of killing in two hours at the temperature of the laboratory the organisms under investigation, suspended either in water or in serum. The technique was as follows:

A series of tubes each containing 5 c.c. of a solution of the substance at a progressively decreasing concentration is first of all prepared, and to each tube one or two drops of a 24-hour-old culture of the organism in peptone bouillon is added. A blank experiment with 5 c.c. of distilled water with a drop of the culture is also made. The mixtures of substance and microbes are carefully shaken every half hour and remain at a temperature of 18–20° for two hours. A loop full of the contents of each tube is then taken and added to a series of tubes, each containing 3 c.c. of bouillon, which are then incubated for 24 hours at 37°. When no growth appears in these inoculated tubes, the concentration of the antiseptic in the first series of tubes is judged sufficient to kill the organisms. Incomplete sterilisation is indicated by growth, and the necessary concentration for sterilisation can be determined more closely by subsequent repetitions at less widely varying strengths of solution.

The tests carried out in the presence of blood serum were performed in the same way, only the liquid contained in the first series of tubes contained 50 per cent. of horse serum previously heated to 55–56°. One drop of culture was added for every 2 c.c. of the serum mixture.

Action of Chloramines on Amino-acids.—The chloramine employed in these experiments was usually *p*-toluene sulphochloramide. When an aqueous solution of this substance is added to a solution of an amino-acid, *p*-toluene sulphonamide is rapidly precipitated, and a pungent odour, probably due to a chloramino-acid, is noted. On standing or on warming the solution, carbon dioxide is rapidly evolved through the decomposition of the chloramino-acid, and an aldehyde is formed. In the following experiments equimolecular quantities of the amino-acid and toluene sulphonamide in water were mixed and then distilled.

Glycine gave formaldehyde, which was characterised by its nitrophenylhydrazone, M.P. 181°, by conversion into hexamethylenetetramine on evaporation with ammonia, and by various colour reactions.

Glycine anhydride behaved similarly to the above.

Alanine gave acetaldehyde, which was identified by conversion into the nitrophenylhydrazone, which, after crystallisation from alcohol, melted at the correct temperature, 130°. The Rimini reaction with sodium nitroprusside and piperidine was strongly positive.

Leucine gave oily isovaleric aldehyde which was identified by conversion into the nitro-phenylhydrazone.

Amino-phenylacetic acid and *methylamino-phenylacetic acid* both gave large quantities of benzaldehyde, which for purposes of identification was converted into the phenylhydrazone, crystallising from alcohol in needles, M.P. 159°, and into the nitro-phenylhydrazone, M.P. 192°. In each case the aldehyde obtained contained one less carbon atom than the amino-acid taken, and it is clear that the reaction proceeds according to the following scheme :—

$$R.CH(NH_2).COOH + CH_3.C_6H_4.SO_2Na : NCl = R.CH(NHCl).COONa + CH_3.C_6H_4.SO_2.NH_2.$$

$$R.CH(NHCl).COONa + H_2O = R.CHO + CO_2 + NaCl + NH_3.$$

The similarity between this reaction and the oxidation of amino-acids with hypochlorite has already been noted.

Tryptophane.—When *p*-toluene sulphochloramide is added to tryptophane a blue-violet substance soluble in amyl alcohol, and closely resembling the coloured product of the Hopkins glyoxylic acid reaction is obtained.

Blood Serum.—When chloramine dissolved in water is left in contact with blood serum at the ordinary temperature for 2–3 weeks and the product distilled, the distillate gives the colour reactions for both formaldehyde and acetaldehyde, and a crystalline phenylhydrazone, melting indefinitely at 135–150°, which is probably a mixture of the phenylhydrazones of the two aldehydes (130° and 178°).

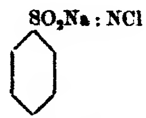
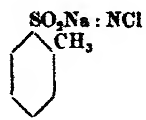
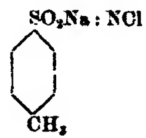
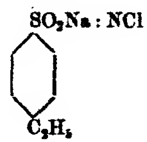
Stability of p-Toluene Sodium Sulphochloramide.—Reference has already been made to the remarkable stability of toluene sodium sulphochloramide in solution as evidence of the non-dissociation of sodium sulphochloramides into sulphonamides and sodium hypochlorite. The following Table contains the results of some of these observations, which were made with *p*-toluene sodium sulphochloramide. This substance was chosen since, on account of its practical applications, the information was of special value. The concentrations of the solutions were determined by titration with decinormal sodium thiosulphate after addition of potassium iodide and acetic acid. It will be seen that the rate of decomposition of the solutions in the dark is inappreciable after 132 days, while solutions exposed to daylight showed a slight but trivial loss of strength. Two other points which may be of practical value may be mentioned. *p*-Toluene sodium sulphochloramide should not be left in long contact with steel instruments, since, as would be expected, the metal is attacked. In order to see if clean surgical gauze would cause decomposition of the sulphochloramide, a solution containing

1·72 grm. in 50 c.c. was added to 1 square foot of gauze. The decomposition was only 0·05 grm. in 123 days, and the gauze itself was not damaged in any way.

Days..............	0.	1.	2.	8.	16.	23.	51.	123.
In light	0·832	0·818	0·812	0·795	0·792	0·782	0·775	0·756
"	1·653	1·639	1·639	1·608	1·596	1·579	1·543	1·540
"	3·282	3·282	3·277	3·225	3·193	3·192	3·086	3·080
In dark	0·826	0·815	0·815	0·815	0·815	0·815	0·815	0·815
"	1·648	1·648	1·648	1·648	1·642	1·642	1·642	1·642
" at 37° C.	1·648	1·648	1·648	1·648	1·648	1·648	1·648	1·648
"	3·311	3·311	3·294	3·300	3·294	3·294	3·311	3·282

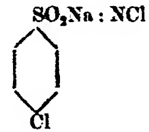
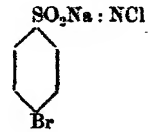
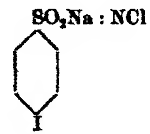
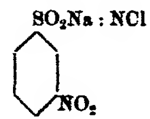
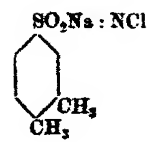
The figures in the above Table represent grammes per 100 c.c. of solution.

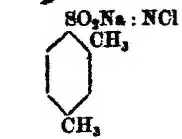
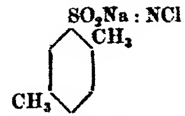
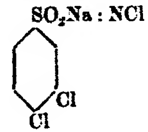
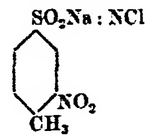
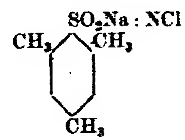
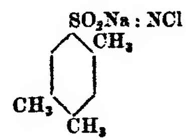
TABLE OF RESULTS.

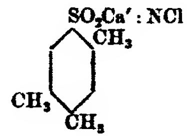
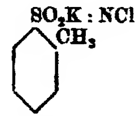
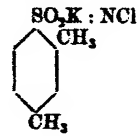
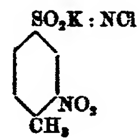
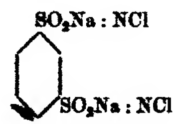
No.	Name.	Formula.	Staphylococci tests.		Pyocyaneus tests.	
			In water.	In serum.	In water.	In serum.
	SERIES I.					
1	Benzene sodium sulphochloramide*		1 : 250,000 – 1 : 500,000 +	1 : 1000 – 1 : 2500 +	1 : 50,000 – 1 : 1,000,000 +	1 : 500 – 1 : 1000 +
2	*o*-Toluene sodium sulphochloramide		1 : 500,000 – 1 : 1,000,000 +	1 : 500 – 1 : 1000 +		
3	*p*-Toluene sodium sulphochloramide†		1 : 500,000 – 1 : 1,000,000 +	1 : 1500 – 1 : 2500 +	1 : 200,000 – 1 : 500,000 +	1 : 1000 – 1 : 2000 +
4	*p*-Ethylbenzene sodium sulphochloramide		1 : 100,000 – 1 : 250,000 +	1 : 1000 – 1 : 2500 +	1 : 100,000 – 1 : 250,000 +	1 : 500 – 1 : 1000 +

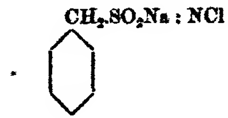
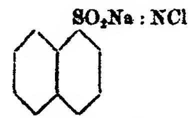
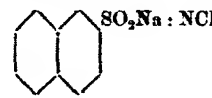
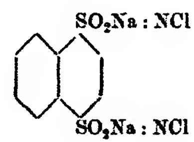
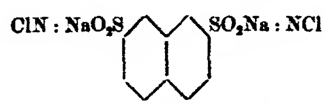
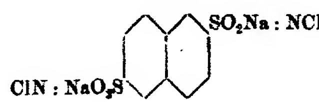
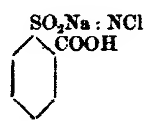
* Tested against streptococci, this substance gives the following results: in water, 1 : 500,000 –, 1 : 1,000,000 + ; in serum, 1 : 1000 –, 1 : 5000 +. Against *B. coli* and *B. typhosus*: in water, 1 : 50,000 –, 1 : 100,000 + ; in serum, 1 : 500 –, 1 : 1000 +.

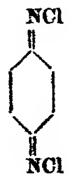
† Streptococci: in water, 1 : 10,000,000 – ; in serum, 1 : 2500 –, 1 : 5000 +. With *B. capsulatus*, the results are the same.

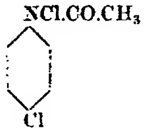
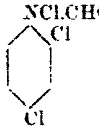
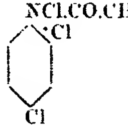
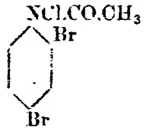
No.	Name.	Formula.	Staphylococci tests.		Pyocyaneus tests.	
			In water.	In serum.	In water.	In serum.
	SERIES I—*continued.*					
5	*p*-Chlorobenzene sodium sulphochloramide		1 : 100,000 – 1 : 250,000 +	1 : 1000 – 1 : 2500 +	1 : 50,000 – 1 : 100,000 +	1 : 500 – 1 : 1000 +
6	*p*-Bromobenzene sodium sulphochloramide		1 : 100,000 – 1 : 250,000 +	1 : 500 – 1 : 1000 +	1 : 10,000 – 1 : 50,000 +	1 : 500 – 1 : 1000 +
7	*p*-Iodobenzene sodium sulphochloramide		1 : 100,000 – 1 : 250,000 +	1 : 1000 – 1 : 2500 +	1 : 25,000 – 1 : 50,000 +	1 : 500 – 1 : 1000 +
8	*m*-Nitrobenzene sodium sulphochloramide		1 : 25,000 – 1 : 50,000 +	1 : 500 – 1 : 1500 +	1 : 10,000 – 1 : 25,000 +	1 : 500 – 1 : 1000 +
9	*o*-Xylene sodium sulphochloramide		1 : 100,000 – 1 : 250,000 +	1 : 500 – 1 : 1000 +	1 : 25,000 – 1 : 50,000 +	1 : 500 +

10	*m*-Xylene sodium sulphochloramide		1 : 100,000 – 1 : 250,000 +	1 : 1000 – 1 : 2500 +	1 : 50,000 – 1 : 100,000 +	1 : 500 – 1 : 1000 +
11	*p*-Xylene sodium sulphochloramide		1 : 50,000 – 1 : 100,000 +	1 : 500 – 1 : 1000 +	1 : 100,000 – 1 : 250,000 +	1 : 250 – 1 : 500 +
12	*o*-Dichlorobenzene sodium sulphochloramide		1 : 100,000 – 1 : 250,000 +	1 : 1000 – 1 : 2500 +	1 : 25,000 – 1 : 50,000 +	1 : 500 – 1 : 1000 +
13	*o*-Nitrotoluene sodium sulphochloramide		1 : 50,000 – 1 : 100,000 +	1 : 500 – 1 : 100 +	1 : 50,000 – 1 : 100,000 +	1 : 500 – 1 : 1000 +
14	Mesitylene sodium sulphochloramide		1 : 100,000 – 1 : 250,000 +	1 : 1000 – 1 : 2500 +	1 : 50,000 – 1 : 100,000 +	1 : 500 – 1 : 1000 +
15	Pseudocumene sodium sulphochloramide		1 : 100,000 – 1 : 250,000 +	1 : 1000 – 1 : 2500 +	1 : 50,000 – 1 : 100,000 +	1 : 500 – 1 : 1000 +

No.	Name.	Formula.	Staphylococci tests.		Pyocyaneus tests.	
			In water.	In serum.	In water.	In serum.
	SERIES I—*continued*.					
16	Pseudocumene calcium sulphochloramide		1 : 50,000 – 1 : 100,000 +	1 : 1000 – 1 : 2500 +	1 : 50,000 – 1 : 100,000 +	1 : 250 – 1 : 500 +
17	*o*-Toluene potassium sulphochloramide		1 : 10,000 – 1 : 100,000 +	1 : 250 +		
18	*m*-Xylene potassium sulphochloramide		1 : 10,000 +	1 : 250 +		
19	*o*-Nitrotoluene potassium sulphochloramide		1 : 50,000 – 1 : 100,000 +	1 : 250 – 1 : 500 +		
20	Benzene sodium disulphochloramide		1 : 100,000 – 1 : 1,000,000 +	1 : 1000 – 1 : 2500 +	1 : 100,000 – 1 : 1,000,000 +	1 : 1000 – 1 : 2500 +

21	Benzyl sodium sulphochloramide		1 : 100,000 – 1 : 250,000 +	1 : 500 – 1 : 1000 +	1 : 50,000 – 1 : 100,000 +	1 : 500 – 1 : 1000 +
	Series II.					
22	α-Naphthalene sodium sulphochloramide		1 : 250,000 – 1 : 500,000 +	1 : 1000 – 1 : 2500 +		
23	β-Naphthalene sodium sulphochloramide		1 : 100,000 – 1 : 250,000 +	1 : 500 – 1 : 1000 +		
24	1 : 4 Naphthalene sodium disulphochloramide		1 : 10,000 – 1 : 50,000 +	1 : 500 – 1 : 1000 +	1 : 10,000 – 1 : 50,000 +	1 : 500 – 1 : 1000 +
25	2 : 6 Naphthalene sodium disulphochloramide		1 : 250,000 – 1 : 500,000 +	1 : 500 – 1 : 1000 +	1 : 250,000 – 1 : 500,000 +	1 : 500 – 1 : 1000 +
26	2 : 7 Naphthalene sodium disulphochloramide		1 : 250,000 – 1 : 500,000 +	1 : 250 – 1 : 500 +	1 : 100,000 – 1 : 250,000 +	1 : 250 – 1 : 500 +
	Series III.					
27	Sodium *o*-sulphochloramidobenzoate		1 : 100,000 – 1 : 500,000 +	1 : 250 – 1 : 500 +		

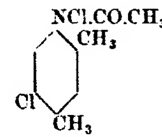
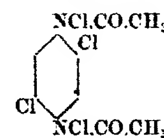
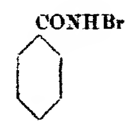
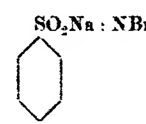
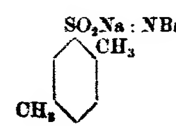
No.	Name.	Formula.	Staphylococci tests.		Pyocyaneus tests.	
			In water.	In serum.	In water.	In serum.
	Series III—continued.					
28	*p*-Diphenyl sodium disulphochloramide*	$C_6H_4.SO_2Na : NCl$ | $C_6H_4.SO_2Na : NCl$	1 : 250,000 – 1 : 500,000 +	1 : 250 – 1 : 500 +	1 : 250,000 – 1 : 500,000 +	1 : 500 – 1 : 1000 +
29	Diphenylmethane sodium disulphochloramide*	$CH_2 \langle {C_6H_4.SO_2Na : NCl \atop C_6H_4.SO_2Na : NCl}$	1 : 250,000 – 1 : 500,000 +	1 : 500 – 1 : 1000 +	1 : 250,000 – 1 : 500,000 +	1 : 1000 – 1 : 2500 +
30	Dibenzyl sodium disulphochloramide*	$CH_2.C_6H_4.SO_2Na : NCl$ | $CH_2.C_6H_4.SO_2Na : NCl$	1 : 50,000 – 1 : 100,000 +	1 : 250 – 1 : 500 +	1 : 50,000 – 1 : 100,000 +	1 : 250 – 1 : 500 +
	Series IV.					
31	Quinone chlorimide		1 : 50,000 – 1 : 100,000 +	1 : 5000 – 1 : 10,000 +		
32	Quinone dichlorimide		1 : 1000 – 1 : 10,000 +			

33	**Acetylchloraminobenzene**†		1 : 100,000 – 1 : 500,000 +			
34	**Acetylchloramino-*p*-chlorobenzene**		1 : 100,000 – 1 : 500,000 +			
35	Formylchloramino-2 : 4-dichlorobenzene		1 : 100,000 – 1 : 500,000 +			
36	**Acetylchloramino-2 : 4-dichlorobenzene**‡		1 : 200,000 – 1 : 500,000 +	1 : 500 – 1 : 1000 +	1 : 500,000 – 1 : 1,000,000 +	1 : 1000 – 1 : 2500 +
37	Acetylchloramino-2 : 4-dibromobenzene		1 : 100,000 – 1 : 500,000 +	1 : 500 – 1 : 1000 +	1 : 200,000 – 1 : 500,000 +	1 : 500 – 1 : 1000 +

* These substances were prepared for us by the kindness of Mr. Joseph Marshall.

† In comparing the results of this and following substances, it should be noted that the related compounds in which chlorine is not attached to nitrogen, namely, formanilide, acetanilide, *p*-chlorformanilide, *p*-chloracetanilide, and 2 : 4 dichloracetanilide show no marked germicidal properties. None of them kill staphylococci in water at much under 1 : 200 concentration.

‡ Streptococci : in water, 1 : 10,000,000 – ; in serum, 1 : 2500 –, 1 : 5000 +.

No.	Name.	Formula.	Staphylococci tests.		Pyocyaneus tests.	
			In water.	In serum.	In water.	In serum.
	SERIES IV—*continued*.					
38	Acetylchloramino-chloroxylene		1 : 100,000 – 1 : 250,000 +	1 : 2000 – 1 : 5000 +		
39	Diacetylchloramino-dichlorobenzene		1 : 100,000 – 1 : 250,000 +	1 : 1000 – 1 : 2500 +	1 : 100,000 – 1 : 250,000 +	1 : 1000 – 1 : 2500 +
	SERIES V.					
40	Benzobromamide		1 : 100 – 1 : 1000 +			
41	Benzene sodium sulphobromamide		1 : 50,000 – 1 : 100,000 +	1 : 250 – 1 : 500 +		
42	*p*-Xylene sodium sulphobromamide		1 : 50,000 – 1 : 100,000 +	1 : 100 – 1 : 250 +	1 : 50,000 – 1 : 100,000 +	1 : 100 – 1 : 250 –

	Series VI.					
43	Chlorinated blood albumin	—	1 : 250,000 – 1 : 500,000 +	1 : 1000 – 1 : 2500 +		
44	Chlorinated egg albumin	—	1 : 100,000 – 1 : 500,000 +	1 : 1000 – 1 : 2500 +	1 : 500,000 – 1 : 1,000,000 +	1 : 2500 – 1 : 5000 +
45	Chlorinated casein	—	1 : 75,000 – 1 : 150,000 +	1 : 1000 – 1 : 2500 +		
46	Chlorinated peptones	—	1 : 50,000 – 1 : 100,000 +	1 : 500 – 1 : 1000 +		
	Series VII.					
48	Sodium hypochlorite	NaClO	1 : 500,000 – 1 : 1,000,000 +	1 : 1500 – 1 : 2000 +	1 : 100,000 – 1 : 500,000 +	1 : 2500 – 1 : 5000 +
49	Sodium hypobromite	NaBrO	1 : 2500 – 1 : 5000 +			
50	Iodine	I	1 : 250,000 – 1 : 500,000 +	1 : 1000 – 1 : 2500 +		
51	Iodine dissolved in weak sodium hydrate	—	1 : 100 – 1 : 250 +			
52	Compound of hexamethylenetetramine and calcium hypochlorite	—	1 : 250,000 – 1 : 500,000 +	1 : 1000 – 1 : 2500 +		

Preliminary Report on the Purbeck Characeæ.

By Clement Reid, F.R.S., and J. Groves.

(Received January 7, 1916.)

[Plate 8.]

When we applied for a grant to aid in the working out of the Purbeck fossil *Characeæ,* we thought it was only a question of studying the anomalous structure of one, or perhaps of two, species, of which we had already gathered together a number of silicified specimens. It was thought that by polishing a large number of surfaces, or cutting slides of this cherty material, we should discover the links connecting the different parts of the plant. Further visits to Dorset provided, however, an enormous amount of new material, and the discovery of similar remains in a hard, close-grained limestone opened up new and better methods of research. The silicified *Characeæ* showed in section curious structures, so mineralised and so difficult to interpret, that it was most desirable to obtain specimens in the round, in order better to study their anatomy. This the calcareous blocks enabled us to do, though, on the other hand, some of the chert specimens preserved delicate non-calcified structures which were missing in the limestone.

On treating some of the limestone blocks with a steady drip of slightly acidulated water, the results were so surprising that we determined to devote most of the amount granted to the work of cutting all the more promising blocks into a series of thin slabs. Numerous slices were cut, and one side of each was then subjected for many hours to the drip. Most of the blocks proved to be partially and irregularly silicified, others were more or less dolomitised, a few were partly impregnated with a brown hydrocarbon. The drip rapidly attacked the pure calcite parts of the matrix and also the crystalline fossils, such as the mollusca, leaving the mineralised areas standing up. This, of course, was what one would expect. But more remarkable was the discovery that a great part of the characeous remains were not now pure calcite. Though not visibly different from the matrix, they are so mineralised as to resist the acid and to stand out in bold relief from the etched surface of the slab of limestone. We have even been able completely to remove fruits and stems from the matrix, though, as a rule, too long a continuance of the acid drip does at last affect the fossils, and it is better to let well alone when a specimen shows in sufficient relief.

The amount of new material thus obtained for the study of the fossil *Characeæ* is so great that it will take us a considerable time to complete the examination and photography. But already we have discovered that, instead of one or two species, there are certainly seven or eight, belonging apparently to four genera. We have that number of markedly distinct types of fruits, and we have about the same number of different types of vegetative organs. This wealth of material is one of the great difficulties in our way. Nearly every good block contains at least three of the types, and these fragile plants have been so broken up and mixed as to be difficult to disentangle. Until we can better correlate the different parts of each plant, and fix more definitely which type of fruit belongs to each, we shall not be satisfied, but meanwhile we will characterise the most abundant species, which is the type of a new and remarkable genus. For this plant we have now obtained the connection of the different parts, and have many specimens with the fruits attached.

The other species are much more rare, though we are gradually obtaining a knowledge of their anatomy. It will be necessary, however, in the coming year to gather more material and to have many more blocks sliced and etched, in the hope that they may give us the connecting links. We think, principally from a study of the association of the forms in each block, that we can with great probability correlate four other types of fruits with the vegetative parts to which they belong, but this correlation would have much greater weight if we could exhibit and photograph the actual attachment of the fruit to the branch, and of the branch to the stem, as can be done perfectly in the type species of our new genus.

Clavator, Reid et Groves (*gen. nov.*).

Caulis simplex, corticatus; axis e cellulis alternatim elongatis et abbreviatis compositus; cortex circa illas tubulosus, circa has fusiformiter tumidus, hoc modo caulem in internodia et nodos quasi dividens; capite tumido turbinato terminatus. *Internodia* e tubo centrali pariete crassa instructa, et cortice semper 12 tubulorum parvorum cylindricorum contiguorum inter se æqualium composita, omnia spatiis subæqualibus interjectis fasciculos symmetricos (rosellas) processuum emittentia. *Rosellarum* processus plus minusve producti, clavati, apice rotundati, quoquoversus divergentes, exteriores ad tubos corticales adnati, processus quisque poro basali cum parte interiore tubi corticalis conjunctus, poris processuum centralium solitariorum vel plurium plerumque majoribus quam processuum exteriorum. *Nodorum capitumque* structura admodum permutata, corticis tubi alternantes sex medium versus cito

ampliati, septis crassis in cellulas distinctas eo breviores quo latiores divisi, sex sensim angustati, extrinsecus propulsi, ideoque in sulcis inter tubos majores positi, omnes rosellas more internodiorum ferentes, majores prætereа ramulos singulos verticillatim dispositos emittentes. *Ramuli* e cellula una tubulari et nonnullis abrupto expansis nodiformibus verticilla singula sex rosellarum more tuborum corticalium emittentibus constantes. *Fructus* solitarii, bini vel terni, uno latere (verisimiliter superiore) ramuli e rosellæ centro (?) orti, oogonia singula quasi bracteata referentes. *Oogonium* compositum ex ovo et ex cellulis 5 elongatis spiraliter tortis, iis Characearum recentium similibus, sed utriculo circumdatum e cellulis bracteiformibus elongatis adnatis effecto vel iis incluso. *Antheridia* ignota.

The principal characteristics of the genus appear to be—

1. The remarkable club-like nodes of the stem, from which we derive the name.

2. The production on the stem and branchlets of clusters of small clavate processes.

3. The presence of a utricle enclosing the oogonium.

The club-like nodes of the stem are of two kinds. The first, which we have styled "spindles" (Plate 8, fig. 13), taper at each end into the normal stem. Although we have not found two of the spindles connected, we conclude, from the fact that they are the more numerous, that each stem produced two or more of them. The second kind, which we have styled "heads" (fig. 5), are terminal and are turbinate, tapering below, and more or less flattened above. The normal stem forming the internodes is composed of a single thick-walled tube, surrounded by twelve equal contiguous sheathing tubes, or series of cells (figs. 9 and 10), resembling the so-called "cortex" of existing Chareæ, except that the number is apparently constant. At the ends of the spindles and at the base of the heads a marked change takes place in the sheathing tubes, six alternate tubes rapidly enlarging and becoming broken up by well-marked transverse septa into separate cells, which diminish in length as they increase in diameter, the other six tubes gradually diminishing in diameter and being forced outwards, so that instead of, as in the internodes, lying side by side with the alternate tubes, they occupy the furrows between their outer curves (figs. 11 and 12).

The clusters of small clavate processes, which we have styled "rosettes," are produced at more or less regular intervals on the sheathing tubes of the stem, both on the nodes and internodes; they are symmetrical, the processes diverging in all directions, rosette fashion, the outer being adnate to the sheathing tube from which they originate. With the interior of the tube

each process is connected by a pore at the base, the central one or more of the pores being larger than the lateral; the processes are more or less elongated, swelling upwards, and are round-ended. Though occupying the same position, these processes are evidently not analogous to the spine-cells of living Chareæ, as they are not separate cells.

The head gives rise to a whorl of six branchlets, which are produced from the broadest part. Similar whorls occur on some (probably the upper) spindles.

The branchlets (fig. 3), which are spreading or ascending, are composed of a single tube, which becomes suddenly swollen at more or less regular intervals, producing whorls of six clusters of processes, somewhat resembling the rosettes of the sheathing tubes of the stem.

The fruits are produced singly, or two or three together, on one, probably the upper, side of the branchlets, taking rise from pores at the centre (?) of the rosettes.

The fruit consists of an oogonium with five spiral enveloping cells, as in existing Characeæ, but enclosed in a utricle formed or surrounded by a number of elongated adnate processes, somewhat similar to the bract-cells of living species of *Chareæ*, converging at the tips and nearly closing in the utricle. Little more than the calcified portion of these bract-like processes adnate to the fruit is preserved (see figs. 1, 2, 4, 6, 7, 8). Up to the present we have not found any trace of the antheridia.

We have no indication as to the stature of the plant, having found short portions only of the stem, and we have not made out any trace of a rooting system.

The foregoing description is drawn up from the remains of what is evidently the commonest species, but we have found others which, though different in some respects, belong apparently to the same type.

In 1891, Saporta, in his 'Plantes Jurassiques,'* described and figured as Chara fruits, under the name of *C. Maillardi*, some grooved pyriform bodies, which may be the utricles of a species of this genus.

The production of the "rosettes" is apparently a character shared by at least one of the other genera. The constant number of the branchlets (six) is probably common to all the Characeæ of these Purbeck beds, as well as the fixed number (twelve) of the sheathing tubes of the stem, when present.

Among the other genera is one evidently belonging to the Nitelleæ, having forked branchlets. This is apparently the first satisfactory instance of a

* 'Paléontolog. Franç.,' ser. 2, Végét. IV, p. 498, tab. 298 *bis*, figs. 6 and 7 (1891).

representative of this section being found in a fossil state. It is silicified not preserved in limestone.

EXPLANATION OF PLATE.

Fig. 1.—Oogonium in utricle.
Fig. 2.—Longitudinal sections of two fruits.
Fig. 3.—Longitudinal section of part of branchlet, showing rosettes and a fruit attached.
Fig. 4.—Three fruits attached to branchlet, showing exterior of utricles.
Fig. 5.—Head, an elongated form, fractured obliquely.
Fig. 6.—Transverse section of fruit, showing portions of adnate bract-processes.
Fig. 7.—Utricle seen from above, with section of apex.
Fig. 8.—Longitudinal section of fruit and part of branchlet (silicified).
Fig. 9.—Transverse section of internode of stem, near thickened node, showing slight difference in size of sheathing tubes.
Fig. 10.—Two portions of stems showing rosettes.
Figs. 11 and 12.—Transverse sections of spindles or heads, showing very unequal diameters of sheathing cells, and the bases of some branchlets.
Fig. 13.—Longitudinal section of spindle, showing base of ascending branch.

Clavator, n.g.

The Endemic Flora of Ceylon, with Reference to Geographical Distribution and Evolution in General: A Correction.

By J. C. WILLIS, M.A., Sc.D., late Director of the Botanic Gardens, Rio de Janeiro.

(Communicated by D. H. Scott, For. Sec. R.S. Received March 21, 1916.)

I much regret that, owing to some unaccountable carelessness on my part, Table IX, p. 315 of a recent 'Phil. Trans.' paper* was printed from my first draft, and not from the final copy. To it there must be added :—

Genus.	Species and Marks.					
	Ceylon.	Rarity.	Ceylon and P. India.	Rarity.	Wider.	Rarity.
Polygala	2/5	2·5	1/4	4·0	5/17	3·4
Grewia	1/5	5·0	4/23	5·7	5/13	2·6
Erythroxylon	2/6	3·0	1/5	5·0	1/1	1·0
Trichosanthes	1/5	5·0	1/3	3·0	2/5	2·5
Psychotria	9/46	5·1	3/4	1·3	1/2	2·0
Gymnema	1/5	5·0	1/4	4·0	2/6	3·0
Cordia	1/5	5·0	1/2	2·0	3/13	4·3
Ipomœa *add*	—	—	2/7	3·5	16/51	3·1
Zingiber	1/2	2·0	1/2	2·0	2/7	3·5
Cyperus	1/6	6·0	3/9	3·0	30/106	2·9
Total	19/85	4·4	18/63	3·5	73/221	3·0

This error, however, affords the opportunity of once more calling attention to the extraordinary regularity with which the figures follow my suggested law of rarity. The total of the omitted genera goes in perfectly regular order, and so large an omission makes no difference to the final result. There are two errors in the total given for Table IX, which should read

310/1367	4·4	166/569	3·4	315/889	2·8

Addition of the omissions given above makes

329/1452	4·4	184/632	3·4	388/1110	2·8

* "The Endemic Flora of Ceylon, with Reference to Geographical Distribution and Evolution in General," 'Phil. Trans.,' B, vol. 206, p. 307 (1915).

The Growth-Rings on Herring Scales.

By Geoffrey W. Paget, B.A., and Robert E. Savage, A.R.C.Sc.

(Communicated by Dr. A. T. Masterman, F.R.S. Received April 1, 1916.)

This note summarises the results of certain investigations on this subject carried out in the Laboratory of the Board of Agriculture and Fisheries. The researches of each author were carried out independently, and their full reports will be published in the Fisheries Investigation Series of Reports of the Board.

The number of scales on Teleostean fish being roughly the same throughout life, it is reasonable to suppose that they follow in their individual growth the growth of the fish as a whole: that when the fish is growing fast the scales grow at a corresponding rate. This differential growth-rate is clearly shown on those scales which are marked with concentric striations, for in these cases the striæ appear closer together when growth is slack than they do when growth is vigorous.

By means of marking experiments, such as have been conducted with salmon, whereby the age of the fish is definitely noted, the alternate close and open growth exhibited by the striæ on the scales have been shown in general to correspond, with some reservations, with the recurring seasons of the year. Hence, in this species, it is possible from observations of the scales to determine, in many instances with a high degree of certainty, the age of the fish. The same method has been applied to other fish, notably the eel, cod, haddock, carp and herring, with varying success. The last-mentioned species has, in recent years, received particular attention; a great mass of data has been collected and conclusions of far-reaching importance deduced.

Between the scales of the salmon and those of the herring, however, a very marked difference exists. Whereas, in the former, the striæ are concentric and thus are capable of reflecting by their grouping the rate of enlargement of the scale as a whole, in the herring, on the other hand, as hitherto described, the striæ are eccentric, and in many cases run in almost straight lines from side to side across the anterior half of the scale. For this reason no differential grouping of striæ can be seen. Instead, at intervals upon the field of the scale, there occur abrupt transparent concentric rings which appear to have no relation whatever with the eccentric striæ. It is, nevertheless, these rings which are regarded as marking recurring periods

of minimum growth corresponding with the winter-season of the year and thus affording an index of the age of the fish.

At present the chief morphological evidence on the point rests upon the observation that, in the majority of cases, scales taken from herring in winter time have what is called a "clear edge"; while those taken from fish in summer appear striated right up to the extreme edge. It is thought, therefore, that the "clear edge" of winter persists in more or less degree and may be identified subsequently as the transparent ring. Not only, however, do many exceptions occur, but it is admitted, by those who have the most thorough acquaintance with scale-reading, that it is not possible to say by inspection whether a transparent ring is in process of formation or no.

It is the purpose of this communication to detail as shortly as possible such additional evidence as we have been able to obtain with respect to the structure and significance of these rings.

Our observations fall under three heads:—

(1) Comparison of dorsal and lateral scales taken from the same fish.

(2) Comparison of the effect produced when the scale is viewed under polarised light with that obtained under ordinary conditions.

(3) The ring as seen in section.

Contrary to common belief, not all the scales of a herring are of the kind described above. If scales be taken from the back, from the region lying between the dorsal fin and the root of the tail, they will be seen to be marked by concentric striæ quite similar in their disposition to those of, say, the salmon. For this reason, such scales indicate clearly a differential growth-rate; further, it can be shown beyond doubt that the limits of the close zones of slow growth coincide in point of position with the transparent rings of lateral scales taken from the same fish. It is fair, therefore, to assume that the transparent ring on the lateral scale does in fact represent a period of minimum growth.

Before discussing our second observation, it should be remarked that the scale of such a fish as the herring is made up of two distinct layers, an upper layer lying unconformably upon a lower, which is itself built up of successive lamellæ added to the inner surface of the scale.

Each lamella, as it is formed, is greater in area than any of those preceding it; and this process coupled with the simultaneous growth in extent of the upper layer brings about the general enlargement of the scale. In section, the upper layer may very clearly be seen lying, unconformably as it were, upon a succession of "outcrops" of the lower. When viewed from above under

polarised light (in conjunction with certain stains) it is this lower lamellar layer (not, as in the previous case, the upper striated layer) which focusses our attention. Under these conditions the scale appears to be made up of a number of concentric bands alternately dark and light.

These bands represent the outcrops of successive lamellæ and are not of equal width but show distinct zoning. Further, if suitable comparisons be made, the limit of the narrow zone will be found to correspond with the transparent ring, just as did the narrow zone of the concentric striæ on the dorsal scales; whilst, in section, the lamellæ which come to the surface at this point not only show very narrow outcrops (as we should expect from our surface view), but show diminished bulk as well. It is clear, therefore, that at the time of the formation of the ridge of the upper layer, the lamellar development of the lower layer was at a minimum.

The physical explanation of the light and dark bands seen under polarised light is to be found in the minute structure of the lamellar layer. The fibres which compose this layer are arranged in two distinct series, one radial, the other concentric; each series is proper to a single lamella, and the lamellæ themselves are arranged in such a manner that the fibres of contiguous lamellæ are at right angles.

As a result of numerous experiments with stained and unstained scales under varying conditions of light, it seemed most probable that the light and dark bands owed their appearance to reflection from the corrugated surfaces of successive outcrops, the corrugations being produced by the individual fibres. That this is the true explanation of the phenomenon is supported by the fact that an effect precisely similar to that of a scale under polarised light may be obtained by engraving upon a piece of copper plate a series of radial and concentric lines in such a manner as to simulate in their arrangement the fibres on the upper surface of the lower layer of a herring scale as ascertained by dissection.

Apart, however, from the physical explanation of the polarisation effect it is obvious that the method affords a most valuable check on the reading of scales by ordinary light and affords important corroborative evidence, when taken in conjunction with that adduced already, of the truth of the view that the transparent rings do indeed mark recurring periods of minimum growth.

On the Classification of the Reptilia.

By Edwin S. Goodrich, F.R.S., Fellow of Merton College, Oxford

(Received May 11, 1916.)

It is gradually becoming recognised that the class Reptilia is not a monophyletic group of diverging forms sprung from a common stem, like the class Aves or the class Mammalia; but is an assemblage containing, on the one hand, the ancestors of the Mammalia, and, on the other hand, the ancestors of the Birds, together with the early Amphibian-like Amniotes, which became adapted to a terrestrial mode of life. In fact, the Reptilia represent not a class but a grade of structure. This group includes a main stem leading from the Stegocephalian type to a central point of divergence of two main branches, one giving rise to the Birds, the other to the Mammals (as shown in the diagram, fig. 1). In addition, there are, of

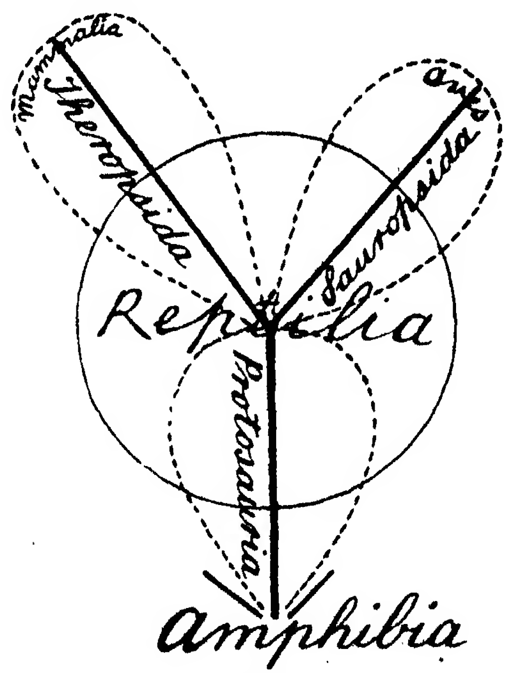


Fig. 1.

course, many abortive side twigs. Some day, no doubt, when the exact relationship of the various living and extinct reptiles has been more accurately determined, it will be necessary to split up the artificial group Reptilia, assigning some to the Mammalia and some to the Aves; but for the

present we may be content with keeping the class Reptilia, always remembering that it is a grade of ill-defined limits.

The modern views of the phylogenetic relationships of the various orders of Reptilia may be said to have arisen chiefly from the work of Cope and Baur, following on the conclusions of Huxley and other earlier authors. To the ingenuity of Cope we owe the valuable suggestion that the starting-point of the divergence between the Amphibia and the Reptilia was determined by the structure of the vertebral column—the vertebral body being mainly derived from the hypocentrum in the former and from the pleurocentrum in the Amniota. Being thus provided with a means of distinguishing the early reptiles from their Amphibian relatives, the next step is to seek for characters enabling us to trace out the diverging lines among the Reptilia themselves. Here again we are indebted to Cope (13), but more especially to Baur (1, 2) for pointing out the importance of the roofing of the skull in classification. Whereas the earliest and most primitive reptiles have, like their Amphibian ancestors, a roofing complete over the temporal region, this becomes pierced in others by one or two foramina. Thus are left one or two longitudinal temporal arches. The formation of the foramina or fossæ is generally accompanied by a reduction in the number of bones covering the hinder region of the skull. It is not my intention to enter into a detailed account of these points in this paper; they have been discussed by many authors, and are well understood. It will be sufficient for our present purpose to point out how profoundly the modern classification of the Amniota has been affected by their recognition.

Owen and Cope long ago drew attention to the Mammalian affinities of certain fossil reptiles now included in the orders Cotylosauria and Theromorpha; while Huxley emphasised the relationship of the birds to the other orders, more especially the Crocodilia and the Dinosauria. Huxley, indeed, included the Reptilia with the Aves in the group Sauropsida, and believed the Mammalia to have been independently derived from more Amphibian-like ancestors. He failed at that time (17) to appreciate the fact that his group Sauropsida included forms, like the Dicynodontia and the Sauropterygia, which belong in all probability to the Mammalian line.

When, chiefly owing to the remarkable discoveries of Seeley and Broom, the true affinities of the extinct Theromorpha became established, the tendency to split the Reptilia into two diverging branches became more pronounced. The importance of the skull-roofing in this connection was appreciated by A. Smith Woodward (37), but it was not till 1903 that Osborn definitely attempted to divide the whole class Reptilia into two

groups which he called the Synapsida (with one lateral temporal fossa and one bar) and the Diapsida (with two lateral temporal fossæ and two bars). This paper marks an epoch in classification. In the Synapsida were placed the Cotylosauria, Anomodontia, Testudinata, and Sauropterygia; in the Diapsida were placed the Protorosauria, Pelycosauria, Rhynchocephalia, Procolophonia, Proganosauria, Choristodera (Simœdosauria), Rhynchosauria, Phytosauria, Ichthyosauria, Crocodilia, Dinosauria, Squamata, and Pterosauria. But, owing to a great extent to the incomplete state of knowledge at the time, this classification has many weak points and requires modification. For instance, it is now generally recognised that the Cotylosauria (and allied Pareiasauria, Procolophonia, and Microsauria) belong to a low grade of reptiles preserving the original complete roof of the skull, and other Stegocephalian characters. Among these primitive forms will perhaps some day be distinguished the ancestors of the more advanced Synapsida and Diapsida; but, for the present, they may be included in a provisional assemblage of early reptiles forming a sub-grade rather than a true sub-order. In the second place, various orders are placed in Osborn's Diapsida which on further evidence seem to belong to the Synapsida—such as the Pelycosauria, Proganosauria, and possibly the Ichthyosauria.

Useful and important as the roofing of the skull is in classification, it is often difficult if not impossible to ascertain for certain its structure in fossils. Moreover, as is always the case when we endeavour to classify by a single character, we are liable to confuse forms in which foramina have begun to appear with others in which they have been or are being secondarily obliterated, and to misinterpret aberrant modifications. Consequently many reptiles have been repeatedly shifted backwards and forwards from the Diapsida to the Synapsida. Any corroborative evidence derived from other parts would therefore be of value as a clue to affinity, and it is the object of this paper to show that such evidence may be found in the structure of the heart and of the skeleton of the hind foot. The former unfortunately only applies to living forms; but the latter is often available even in fragmentary fossils.

For the purpose of facilitating description, and of making our results clear, the grade or class Reptilia may be provisionally subdivided into three groups (fig. 1):—In the first, which may be called the Protosaurian group, we place the primitive forms connected with the Amphibia and leading on from them to the central point of divergence of the Synapsida and Diapsida, together with other side branches. The Protosauria, then, include the Microsauria, Cotylosauria, Pareiasauria, and Procolophonia (see p. 269). The second group, in which becomes developed one lateral temporal foramen

limited below by a single bar, includes those reptiles which lead towards the mammals, together with side branches. These synapsidan reptiles and the Mammalia make up a monophyletic offshoot to which the name Theropsidan branch may be applied. Lastly, the majority of the reptilian orders belong to the third group, culminating in the Aves, and in which two lateral temporal foramina and two bars are developed. These are the Diapsidan reptiles, and together with the Aves they form the great Sauropsidan group. This attempt at a phylogenetic classification of the Amniota may be expressed in diagrammatic form as shown in fig. 1. In these general conclusions most zoologists would now concur; it is only when we try to assign certain families and orders to definite positions in the system that serious differences of opinion arise. We can now pass to the evidence on which these conclusions are founded.

The Significance of the Metatarsals in Reptilian Phylogeny.

On examining the skeleton of the hind foot of a typical Lacertilian, such as the Iguana shown in fig. 2, C, it is at once seen that, whereas the first four metatarsals are of the normal elongated and straight shape, the fifth metatarsal is quite peculiar, and differs from the others in that it is shortened and markedly hooked. The bent proximal end projects forwards (inwards), and also extends farther proximally than the remaining metatarsals, passing over the end of the fourth. This peculiar shape and disposition of the fifth metatarsal has, of course, been often noticed by anatomists, but its significance in classification seems not to have been fully appreciated. Much controversy has taken place about the exact homology of the hook-shaped bone. Some believe it to represent the modified fifth distal tarsal, others the fifth metatarsal combined with its tarsal, and yet others that it represents the modified fifth metatarsal only. This last interpretation is the one now generally accepted, and is clearly shown in Sewertzoff's excellent account (31) of the development of Ascalobotes (see fig. 2, D, E). The question of its homology scarcely concerns us here; the important point to establish for the purpose of this paper is that this peculiarity is found in the hind foot not only of all known Lacertilia, but also of all living reptiles. So far as I can ascertain, it occurs in all Chelonia, Rhynchocephalia, Crocodilia, and Lacertilia, excepting, of course, in those forms which have lost the hind limb. Examples are shown in fig. 2. This particular specialisation of the fifth metatarsal is therefore far more constant than the structure of the skull. Once acquired it never seems to be lost, and it can still be clearly seen even in the feet of the Chelonidæ or the Pythonomorpha, which have become so highly modified into swimming paddles. The hook-shaped metatarsal does

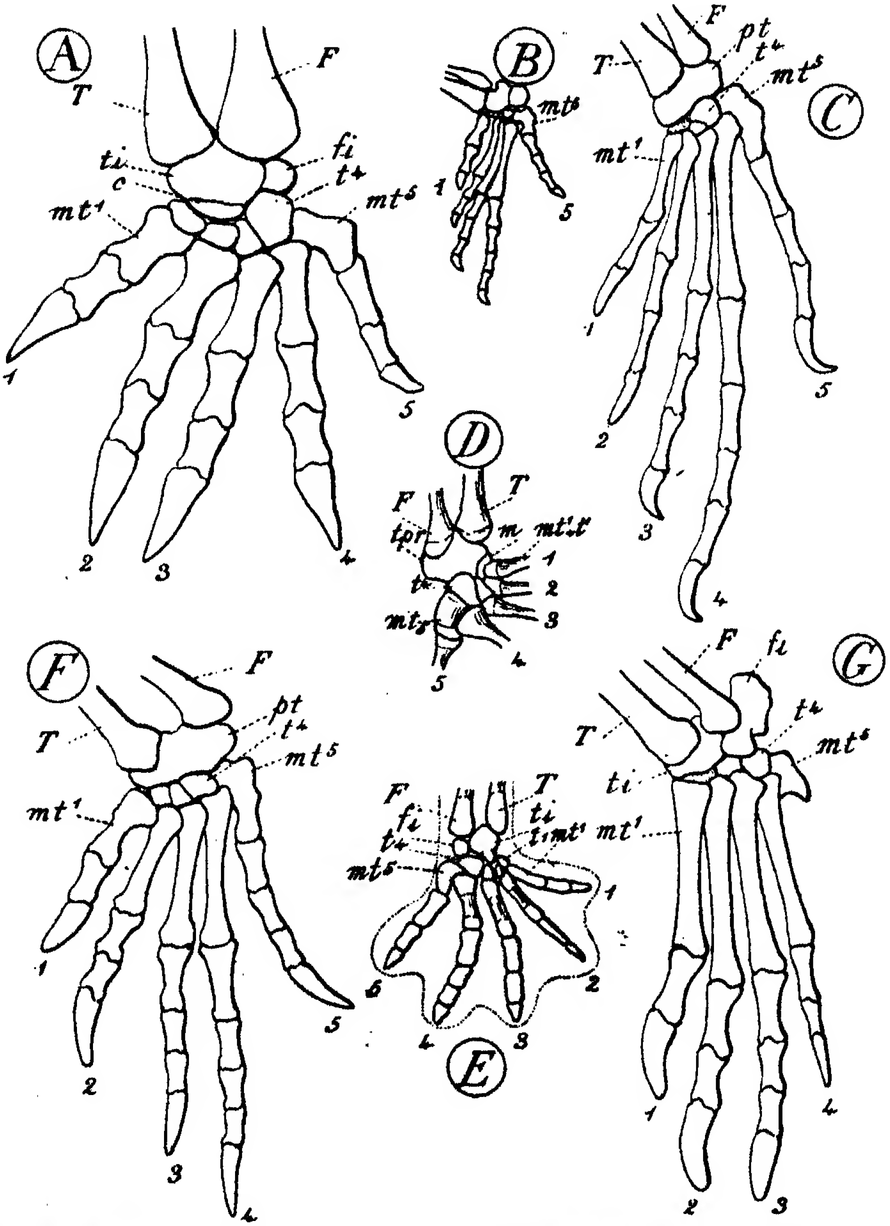


FIG. 2.—Skeleton of the Hind Foot of :—A, *Chelydra serpentina*, L. ; B, *Sauranodon* (*Saphaeosaurus*) *incisivus*, Jourdan ; C, *Iguana tuberculata*, Laur. ; D, *Ascalobotes fascicularis* ; E, Embryo of same species ; F, *Sphenodon punctatus*, Gray ; G, *Caiman sclerops*, Schn. D and E copied from Sewertzoff (31), B from Lortet (21), A, C, F, G, from photographs of specimens in the Oxford University Museum.

not seem to be closely related to any particular mode of life or method of progression, being essentially the same in reptiles of the most diverse habits. Its development is possibly correlated with the formation of the mesotarsal articulation so characteristic of the Sauropsidan reptiles and birds. It is also accompanied by the disappearance of the fifth distal tarsal. Since there is no reason whatever to suppose that it has arisen independently in the various orders, we can only assume that the hook-shaped metatarsal was present in the common ancestor of all the forms which possess it. The mesotarsal articulation, above referred to, is due to the close connection or fusion of the proximal tarsals with the tibia and fibula, and the distal tarsals with the metatarsals.

Now, in the Mammalia, the fifth metatarsal is of normal structure. When the fifth digit is not reduced its metatarsal is straight, and articulates with the cuboid (fourth and fifth distal tarsals fused) at the usual level. We should not therefore expect to find a hook-shaped metatarsal in any fossil reptile leading towards the Mammalia, and this expectation is fulfilled, since there is no trace of it in the Theromorpha.

Of the remaining Reptilia with one fossa and bar, the Ichthyosauria unfortunately yield no certain evidence, since their foot is too modified. But the more primitive Sauropterygia, such as the Lariosauridæ, clearly display a normal fifth metatarsal (fig. 3, F), thus confirming the view, which is now gaining ground and based on other evidence, that the Sauropterygia are allied to the Theromorpha. This view is, of course, incompatible with that of Jaekel, who believes the Sauropterygia to be Diapsida which have lost the lower temporal bar.*

Also, if our contention is correct, that the modified fifth metatarsal is a specialisation occurring only in that line of reptilian phylogeny leading towards the Birds, we should expect to find it absent in all the Cotylosauria and allied Protosaurian forms. Here, again, the facts support our view, for these early reptiles have normal metatarsals like their Amphibian ancestors.

It is clear, then, that we have here a valuable corroborative character to help us to decide whether a given species belongs to the Theropsidan or the Sauropsidan line of evolution. It will not be necessary in this paper to give a description of the metatarsus of all known living and extinct Reptiles; the results of my investigations, based as far as possible on the examination of actual specimens, but also to a great extent on the published figures and descriptions of others, are summarised on page 274. But it is interesting to consider briefly certain important genera and larger groups whose position

* 'Zool. Anz.,' vol. 35, 1909.

has hitherto been very uncertain, and in which the structure of the skull fails to yield decisive evidence.

Mesosauria (Proganosauria).—The very ancient fossils, *Mesosaurus tenuidens*, Gervais, from South Africa, and the closely allied *Stereosternum tumidum*, Cope, from South America, were placed in a new order Proganosauria by Baur (1), who believed them to be related to the Rhynchocephalia. Osborn

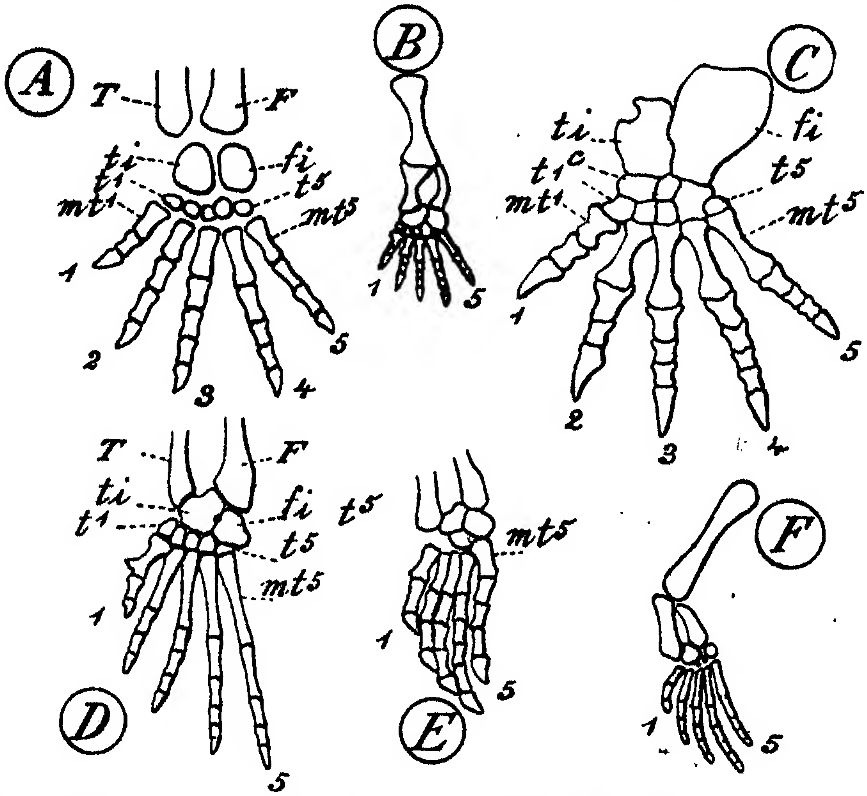


FIG. 3.—Skeleton of the Hind Foot of :—A, *Palæohatteria longicaudata*, Cr., from Credner (14); B, Procolophon, from Watson (33); C, Naosaurus, from Osborn (27); D, Mesosaurus (Stereosternum), from a specimen in the British Museum; E, *Pleurosaurus goldfussi*, H. v. M., from Lortet (21); F, Lariosaurus, from Boulenger (6). All, except E, are somewhat restored.

included them in the Diapsida (26); and in a more recent paper, McGregor, (23), on what appear to be very insufficient grounds, claimed that Mesosaurus has a skull with two temporal fossæ, and is allied to the Rhynchocephalia and Protorosauria (including Palæohatteria). He denies the affinity with the Plesiosauria upheld by Seeley (29), and afterwards so well supported by Boulenger (6). Williston refuses to commit himself (34), but inclines to the view that the Mesosauria are allied to the Theromorpha.

or possibly independently derived from some Cotylosaurian. Broom's observations on the South African fossils (8) seem to lead him to the opinion that they belong to the Diapsida, but yet possessed only one temporal fossa, while von Huene insists on the presence of one fossa only. An examination of the excellently preserved specimen of Stereosternum in the British Museum (fig. 3, D) shows us at once that it cannot be closely allied to the Rhynchocephalia or any known Sauropsidan. Not only is it remarkable in the possession of five distinct distal tarsals, but also in the absence of a mesotarsal articulation, and in that the fifth metatarsal is the longest of all and of quite normal shape. Since we know that the primitive Sauropterygia, such as Lariosaurus, had a normal fifth metatarsal, the view of Seeley and Boulenger seems to be by far the best established.

Palæohatteria.—The history of this genus is instructive. First described by Credner (14) under the impression that it is closely related to Sphenodon, it was credited with a skull provided with two lateral temporal foramina, and for long figured in text-books and other writings as a typical Rhynchocephalian. Baur placed Palæohatteria in his order Proganosauria (3), including it in the Protorosauridæ, which he placed with the Mesosauridæ and Champsosauridæ in the Proganosauria as a sub-order of the Rhynchocephalia. Subsequently Williston (34) dwelt on the affinity of Palæohatteria with the Theromorphs, threw doubt on Credner's reconstruction of the skull, but included this genus together with Protorosaurus in one sub-order Protorosauria. In a later paper (36) Williston gave further arguments for his view, and strongly urged that there is no evidence that either in Palæohatteria or in Protorosaurus there was more than one pair of temporal fossæ, thus agreeing with von Huene.

The hind foot of Palæohatteria (fig. 3, A), provided with a normal elongated fifth metatarsal; shows clearly that it cannot belong to the Rhynchocephalia; rather should it be classified in the Saurotherian group with other reptiles having a single temporal fossa.

Protorosauria.—The important genus Protorosaurus, first described as a crocodile by Spener in 1710, was placed by Seeley in a special order of doubtful affinity but not far from the Dinosauria (28). Unfortunately the skull is not thoroughly known, and it has not yet been determined whether both superior and inferior temporal foramina were present. Long ago, however, it was shown by von Meyer (24) that the hind foot of Protorosaurus is provided with the characteristic Sauropsidan hook-shaped metatarsal, so that we may take it as established that this genus, about whose systematic position there has been so much speculation, is related to the primitive Rhynchocephalia, Crocodilia, or Dinosauria, and has no connection with the Mesosauria.

Related also to these Sauropsidan reptiles would appear to be the Aetosauria or Pseudosuchia (v. Huene, 16), the Parasuchia (McGregor, 22), the Simœdosauria (Lemoine, 20; Brown, 10), and the Rhynchosauria (Huxley, 18), since they all have upper and lower temporal foramina and a hook-shaped fifth metatarsal. On the other hand, the Procolophonia, believed by Broom to have Rhynchocephalian affinities, and placed by Osborn in the Diapsida (26), are now known to have neither temporal fossæ nor a modified metatarsal. For the present they may be placed in our Protosaurian group, and are probably related to the Paraiasauria and Cotylosauria as maintained by Boulenger (7) and Seeley (30).

Pelycosauria.—Founded by Cope in 1878 for certain North American fossil reptiles, such as Dimetrodon and Clepsydrops, supposed to be allied to the Rhynchocephalia, the systematic position of this order has been a subject of much controversy. Cope soon changed his mind and, struck by the resemblance of the Pelycosauria to the South African Theromorpha, believed them to have mammalian affinities. In 1897, however, Baur and Case (4) thought they had proved the existence of upper and lower temporal fossæ, and later the Pelycosauria were included in the Diapsida by Osborn (26). Subsequently Case in his valuable revision of the Pelycosauria (11) still insisted on their close relationship with the Rhynchocephalia, Proganosauria, and Protorosauria. Now the Proganosauria (Mesosaurus and Palæohatteria) we have already seen are probably not Diapsidan, and Broom has recently brought forward strong evidence that the skull of the Pelycosauria is really built on the Synapsidan plan (9). Cope's later opinion is thus borne out, and is certainly more in agreement with the fact that the foot has five normal metatarsals, as described and figured by Case (11) and Osborn (27), see fig. 3, C.

Aræoscelis.—Under the name Aræoscelis Williston has recently described (35) an interesting Permian reptile of lizard-like shape. It is provided with single upper lateral temporal foramen and a broad arch below. These and other characters lead him to suppose that the Lacertilian skull has been evolved from some such form by the narrowing of the arch and the loosening of the quadrate. But if the figure given of the hind foot (36, fig. 5K), and the restoration (36, fig. 7) correctly represent the fifth metatarsal as an elongated straight bone, there can be little doubt that Aræoscelis is not closely related to any modern reptilian order. Rather would it seem to belong to the Theropsida; unless perchance it is an early representative of the Sauropsida before the metatarsal had become modified.

Pleurosaurus.—Watson, who has recently redescribed the skull of *Pleurosaurus goldfussi*, V. Meyer (33A), supports Boulenger's contention (5) that it is not Rhynchocephalian, restores it with one temporal foramen and

one broad bar, and maintains that it is a little modified descendant of the ancestral lizard stock. Believing that the Lacertilia have been derived from such Synapsidan forms, he would separate them from the Rhynchocephalian and other Diapsidan orders. According, however, to Lortet's description and figures (21), one of which is reproduced here (fig. 3, E), the fifth metatarsal would appear to be of normal shape and size. If this interpretation is correct the evidence is distinctly against the view that Pleurosaurus is closely related to the ancestral lizard.

The Significance of the Heart and Aortic Arches in the Phylogeny of Reptiles.

Turning to the evidence to be derived from the structure of the heart and aortic vessels we shall find that it points unmistakably to the very same conclusion reached from a study of the hind foot, namely, that all the modern Reptilia have been derived from a single Sauropsidan branch, distinct from that which led to the Synapsidan Reptilia and the Mammalia.

It is well known that the heart of all reptiles (excepting the Crocodilia) is possessed of a single ventricle, that the cavity of this ventricle is incompletely subdivided by an incipient septum which is only completed in the Crocodiles, and that the four-chambered heart of the latter is essentially like that of a bird. In the possession of two completely separated ventricular chambers the heart of a bird resembles that of a mammal; and it is commonly stated that the two groups differ in that whereas in the former the aortic arch remains on the right side, in the mammal it is the left aortic arch which alone persists. But the difference is far more fundamental than such a statement implies.

The original aortic system, as shown by a comparison of the fish and the embryonic stages of the Tetrapods, consisted of six paired aortic arches. The first supplied the mandibular bar, the second the hyoid, and the remaining four the branchial bars. The last of these arches, the sixth of the original series, gives rise to the pulmonary artery. The heart itself consisted of a series of chambers: the posterior sinus venosus receiving the great veins; the atrium, which in air-breathing vertebrates becomes separated into two auricles; and a ventricle passing forward into a ventral aorta. This trunk becomes divided into a posterior contractile conus arteriosus, or bulbus cordis, and an anterior non-contractile truncus arteriosus, from which spring the aortic arches. The heart becomes twisted, so that the auricles come to lie dorsally and in front of the ventricle; but in the accompanying diagrams (fig. 4) of the heart and arches of an Amphibian (A), a Mammal (B), a Reptile (Lacertilian, Ophidian, Rhynchocephalian, or Chelonian) (C), and a

Crocodile (D), the hearts are represented as untwisted, so as to bring the chambers back into a single plane and facilitate comparison.

Now in the Amphibian a horizontal septum grows back, subdividing the lumen of the truncus into dorsal and ventral channels, and combines with one of the distal valves to form an oblique septum in the bulbus cordis, which septum directs most of the arterial blood into the ventral channel leading to the systemic and carotid arches, and most of the venous blood into a dorsal channel to the pulmonary arches. In the Amniota the valves are fully developed only in that narrow posterior region at the base of the bulbus, which becomes incorporated into the wall of the ventricle. The lumen of the truncus and bulbus becomes completely and spirally subdivided into two tubes, the pulmonary and the systemic or aortic. As shown by the work of Greil (15) and others, this is brought about by the completion of the horizontal septum with the help of the anterior valves of the bulbus. But whereas in the Mammalia the interventricular septum is so formed that the right (venous) ventricle leads only into the pulmonary artery, and the left (arterial) only into the aortic arch and carotids, in the Reptilia the interventricular septum tends to divide the chamber into a left cavity leading to the base of the right systemic arch, and a right cavity leading to the base not only of the pulmonary, but also of the left systemic arch. Thus, when the septum is completed, as in Crocodiles and Birds, the right ventricle opens into the pulmonary artery and left systemic arch, while the left ventricle opens into the right systemic arch. The two systemic arches cross over at their base, and the main arterial stream is always sent up the right arch, from which spring the carotids. The fundamental difference lies in the subdivision of the Sauropsidan bulbus down to its very root into two separate spirally twisted tubes, one crossing to the left and the other to the right, in such a way that the interventricular septum comes to pass between them. This line of specialisation inevitably leads in the long run to the Avian type, where the left systemic arch—already of little use in the Crocodile—disappears early in development. In the Mammal, on the contrary, the aortic trunk, separated from the pulmonary, never becomes subdivided at all, and the differentiation of the arteries has followed an independent and in many respects different course from the primitive bilaterally symmetrical pattern. The Theropsidan and the Sauropsidan types must have evolved from some more symmetrical primitive type, in which the ventricle and the aortic trunk were both single; and it does not seem possible for a heart which had once started, so to speak, to evolve along the Sauropsidan line to change its course and revert to the Theropsidan. The significance of this in determining the phylogeny of the Chelonia and the Lacertilia may now be pointed out.

Chelonia.—Opinions as to the affinities of the Chelonia have been widely divergent. Palæontology affords little or no evidence concerning the origin of this very isolated and specialised order. While some authors have pointed out resemblances to the Rhynchocephalia, Baur (1) and others have held that they are related to the Sauropterygia. Jaekel (19) has derived them from

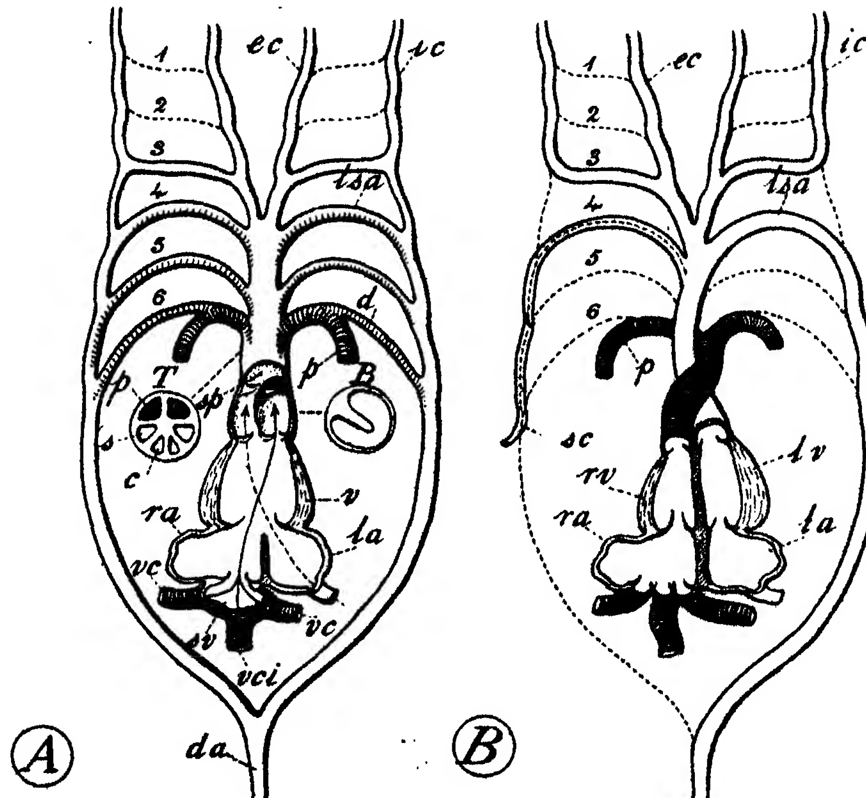
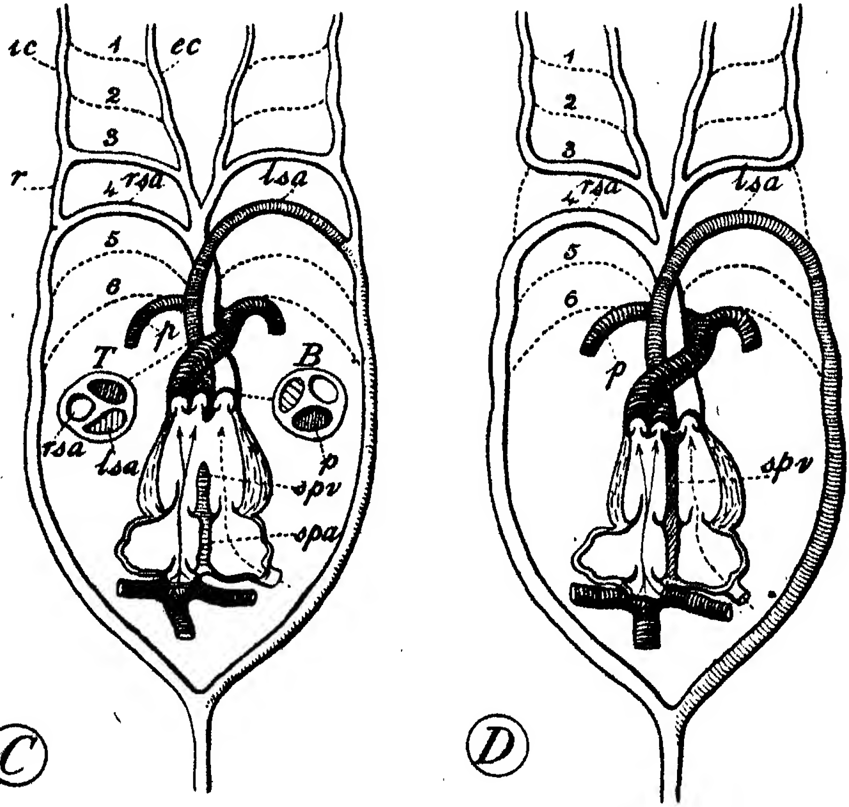


FIG. 4.—Diagrams of the Heart and Aortic Arches of an Amphibian (A), a Mammal (B), a Reptile (Chelonia, Lacertilia, Ophidia, Rhynchocephalia) (C), and a Crocodile (D); ventral view. The heart is represented as untwisted so as to bring the chambers into a single plane, with the sinus venosus behind and the ventricle in front. B, transverse section through region of the bulbus cordis; T, transverse section through the truncus arteriosus; *d*, ductus Botalli; *ec*, external carotid; *ic*, internal carotid; *la*, left auricle; *lsa*, left systemic arch; *lv*, left ventricle; *p*, pulmonary

Placodontia; but most recent writers prefer to derive them independently from some primitive Cotylosaurian ancestor, owing to the absence of true foramina in the temporal roof of the skull. Now the structure of the foot, provided with the characteristic hook-shaped metatarsal, and of the heart built on the Sauropsidan plan, clearly show that the Chelonia belong to the

Sauropsidan branch. There seems to be no escape from this conclusion; nevertheless, it does not solve the difficult problem of the true nature of the roofing of the skull. For it may still be held that the Chelonia branched off from the base of the Sauropsidan stem when the heart and metatarsal had become specialised but the roofing had not yet been pierced. On the whole,



artery; *r*, connecting region which remains open only in Sphenodon and certain Lacertilia; *ra*, right auricle; *rsa*, right systemic arch; *rv*, right ventricle; *sc*, subclavian; *spa*, interauricular septum; *spv*, interventricular septum; *sv*, sinus venosus; *v*, ventricle; *vc*, vena cava superior; *vci*, vena cava inferior. Arrows from the sinus venosus indicate the main stream of venous blood; arrows with a dotted line indicate the stream of arterial blood from the left auricle; 1–6, the original series of six aortic arches.

it seems more likely that the roofing of the skull has been secondarily restored, but the discovery of fossil intermediate forms alone can settle this question.

Lacertilia.—There is much difference of opinion as to the position of the order Lacertilia (including the Pythonomorpha and Dolichosauria). Some,

following Huxley (17), believe that the single temporal fossa of the lizard represents the upper fossa of Sphenodon limited below by the narrow upper temporal bar, while the lower temporal fossa is supposed to have become opened out in the dry Lacertilian skull, the lower bar being represented by a quadrato-jugal ligament. According to this view the Lacertilia are modified Diapsida. Many authors, however, including Baur (2), Williston (35), and Watson (33A), have held that the Lacertilia never had two fossæ, and that their skull is, in fact, built on the same plan as that of the Synapsidan Saurotheria (see p. 269). Here, again, an examination of the hind foot and heart (see pp. 264 and 270) shows us at once that the Lacertilia must belong to the Sauropsidan branch. The characteristic mesotarsal articulation and hook-shaped fifth metatarsal are typically developed, the aortic arches are separated and spirally crossed in the Sauropsidan manner. Palæontology can alone afford convincing proof as to the history of the skull, but the balance of evidence seems to be in favour of Huxley's view.

List of Some Genera Known to Have Normal Metatarsals.—Mesosauria—Mesosaurus (including Stereosternum). Nothosauria—Lariosaurus (Boulenger). Palæohatteria (Credner). Pelycosauria—Varanosaurus (Williston), Dimetrodon, Ophiacodon (Case), Naosaurus (Osborn), Casea (Williston), Aræoscelis ? (Williston), Pleurosaurus ? (Lortet).

List of Some Genera Known to Possess a Modified Fifth Metatarsal.—Chelonia—all living and extinct genera. Lacertilia—all living and extinct genera. Rhynchocephalia—Sphenodon, Homœosaurus (Lortet), Saphæosaurus (Gervais), Rhynchosaurus (Huxley), Simœdosaurus (Lemoine), Champsosaurus (Brown). Protorosaurus (H. v. Meyer). Pythonomorpha—Tylosaurus (Osborn), Mosasaurus (Dollo), Aetosauria—Aetosaurus (v. Huene). Parasuchia—Rhytidodon (McGregor). Crocodilia—all living and extinct genera. Also present, but in a reduced condition, in Dinosauria and Pterosauria.

Summary.

The group Reptilia represents not a true monophyletic class like the class Mammalia and the class Aves, but rather an assemblage or grade of Amniotes retaining a more primitive general structure. The Reptilia thus include a basal *Protosaurian* group of amphibian-like forms leading to a central point from which diverge two main branches—the *Sauropsidan* branch leading to the birds, and the *Theropsidan* branch leading to the mammals.

The modern classification of the reptiles, based chiefly on the structure of the skull, is in a very uncertain state. There is a great difference of

opinion as to the relationship of the various orders. Certain specialisations in the skeleton of the hind foot and in the structure of the heart and great vessels (in living forms) are of great importance in classification and deserve more weight than has hitherto been attributed to them.

The development of a hook-shaped fifth metatarsal and of a mesotarsal articulation, and the sub-division of the aortic trunk so as to form two systemic arches crossing at their base in such a way as to become separated by the interventricular septum, clearly distinguish the Sauropsidan from the Theropsidan line of evolution. The possession of these characters shows that all living Reptilia belong to the Sauropsidan group, while the structure of the foot enables us to determine the affinities of many incompletely known fossil genera, and to conclude that only certain extinct orders can belong to the Theropsidan branch.

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The Relation of Excised Muscle to Acids, Salts, and Bases.

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(From the Zoological Laboratory, Cambridge.)

CONTENTS.

The Influence of the Acidity (or Alkalinity) of the Medium on the Final Equilibria.

The observation that both acids and alkalies cause swelling in excised muscles, and that this swelling is suppressed by the addition of neutral salts, is no new one. The pernicious influence of acids on muscle is referred to as a commonplace by Ringer in 1883 (20, 21) and quantitative data of both acid and alkaline swelling are given by Loeb in 1897 (11). Nevertheless, the subject has never yet received systematic investigation at the hands of any one worker. The following record of experiments showing the behaviour of excised muscle in a range of solutions from decinormal alkali to decinormal acid is intended as a contribution towards filling this vacancy.

An account of some preliminary experiments on the swelling of excised muscle has already been published (9). The method of work in the present case is precisely the same. The sterno-cutaneous muscle of the frog was used in all experiments, and the temperature was kept constant at 20° C. in a thermostat.

Excised muscles placed in acid solution swell rapidly. In hydrochloric acid, which was used in my experiments, the maximum swelling is at a concentration 0·005 normal. This concentration corresponds to a hydrogen ion concentration of $10^{-2.3}$ grm. per litre of solution. At concentrations less than 0·005 normal the degree of swelling diminishes with decreasing concentration, reaching a minimum at the neutral point, *i.e.* in distilled water. At

greater concentrations than 0·005 normal the swelling diminishes with increasing concentration. This is shown in fig. 1.

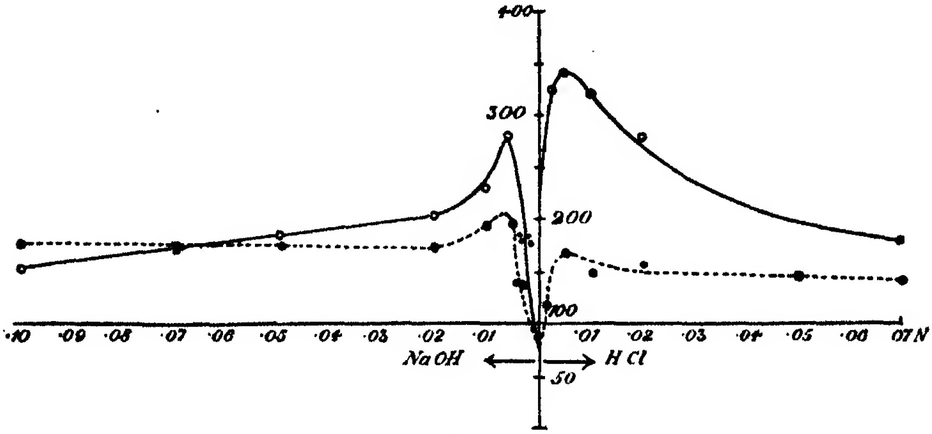

Fig. 1.

Abscissæ = acidity and alkalinity expressed as fractions of normal.
Ordinates = final weights expressed in percentages of initial weights.
The continuous line curve is for acid or alkali in distilled water, the dotted curve is for acid or alkali in Ringer's solution.

In the alkaline solution used (caustic soda) the maximum swelling is also at 0·005 normal. This corresponds to a hydrogen ion concentration $C_H = 10^{-11\cdot7}$ (*i.e.* $10^{-11\cdot7}$ grm. hydrogen ion in 1 litre of solution). The degree of swelling in alkali is not so great as for the corresponding acid. The curve of swelling in alkali falls sharply both above and below the concentration 0·005 normal, as is also shown in the same figure.

The Behaviour near the Neutral Point.

Fig. 1 is a graphical representation of the swelling of muscle in acid or alkali of varying strength. The percentage increase over the original weight is plotted as the ordinate, and the normality of the solution used as the abscissa. It can be seen from the figure that the greatest changes in the degree of swelling occur over a very narrow limit of concentration.

The point of minimal swelling is drawn, in fig. 1, at the point of absolute neutrality for the external medium. Both to the right and left of this point the curve rises very rapidly. In fact, by using the "normality" of the medium as the abscissa to the curve, and the degree of swelling as the ordinate (as has been done in fig. 1), it can be seen, merely by studying the form of the curve, that very minute changes in the concentration of the hydrogen ion present in the external medium must correspond to large changes in the

final equilibrium of the muscle system. It therefore seemed necessary, in order to obtain an accurate record of the phenomena exhibited in an excised muscle in a medium with a reaction near the neutral point, to use some more accurate method of controlling and measuring the acidity or alkalinity of the experimental fluid. The scale obtained by taking "normality" as a measure of acidity is not sufficiently fine to investigate the muscle behaviour at a critical point such as the neutral point of the medium, or the iso-electric point for the muscle. It was, therefore, decided to investigate this critical zone by means of Sörensen's solutions (24). This method not only gives a delicate means of adjusting the hydrogen ion concentration of a solution to any desired value, but also, by means of the "buffers," keeps this value steady when foreign bodies (such as muscles) are put into the system.

In Sörensen's papers there are two different units which have been used to express the concentration of hydrogen and hydroxyl ions in a system. The units C_H or C_{OH} mean respectively the number of grammes of free hydrogen ion or hydroxyl ion present in a litre of solution. They are always given as powers of the base 10, and always have, for obvious reasons, a negative sign to the index. The other units used, expressed by the general term P_H, are the logarithms of the values C_H and, for general convenience, the negative sign is usually omitted. In distilled water $C_H = C_{OH} = 10^{-7 \cdot 1}$, or $P_H = 7{\cdot}1$, and $P_{OH} = 7{\cdot}1$. Now in any solution $P_H + P_{OH} = 14{\cdot}2$, therefore, as P_H increases, P_{OH} decreases. From this it follows that if in any solution $P_H < 7{\cdot}1$, the hydrogen ions predominate and the solution is acid; if $P_H > 7{\cdot}1$, the hydroxyl ions predominate and the solution is alkaline.

The curves obtained by measuring the reaction of a solution by Sörensen's system, and plotting this value against the final equilibrium weight of a muscle immersed therein, are given in fig. 2. The curves in both figs. 1 and 2 are of the same type, *i.e.* they are curves obtained by plotting acidity (or alkalinity) against final equilibrium. They differ in their method of measuring acidity. In fig. 1 acidity is measured on the comparatively rough scale of "normality," *i.e.* on the titration value of the solution. In fig. 2, the actual concentration of the hydrogen ion in the solution is taken as the measure of its acidity. As the points $-0{\cdot}01$ N and $+0{\cdot}01$ N in fig. 1 correspond to the points $P_H = 11$ and $P_H = 3$, respectively, in fig. 2, it can be seen that the critical zone on the curve in fig. 1 has been expanded over a considerably wider area in fig. 2, and can therefore be studied with correspondingly greater accuracy.

The data for making the Sörensen solutions were taken from the chart figured by Walpole (25). The acetate, phosphate, and borate mixtures were used, and by this means a range of P_H from 3 to 11 was examined. The

results of experiments on the degree of swelling in the different solutions are given in fig. 2. It will be noticed that the degree varies according to

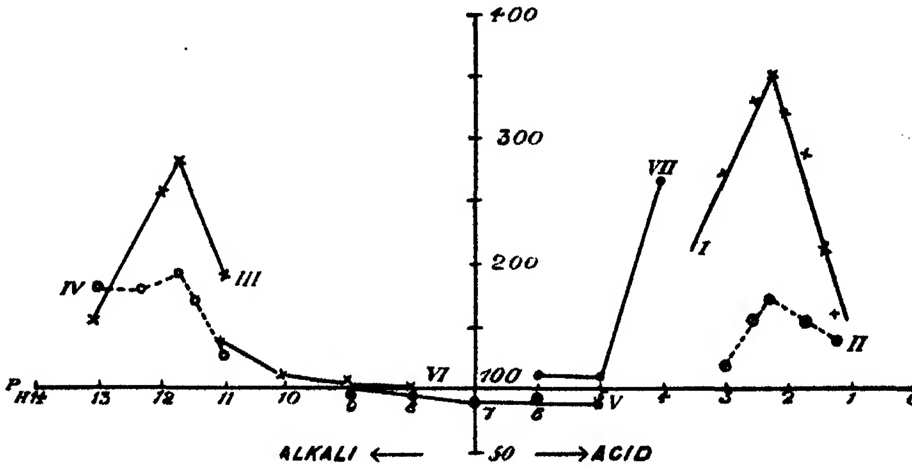

FIG. 2.

Abscissæ = negative logarithm of hydrogen ion concentration.
Ordinates = final weights expressed in percentages of initial weights.

I = curve in HCl; II, HCl + Ringer's solution; III, in NaOH; IV, in NaOH + Ringer; V, in $Na_2HPO_3 + KH_2PO_3$; VI, in Na_2HBO_3 + HCl or + NaOH; VII, in NĀ + HĀ.

the buffer used, the curve for the acetate mixture lying well above the curve for the phosphates. This observation is in line with Bechhold's statement that the phosphates have a greater power of reducing the swelling of gelatine than the acetates. It should be noted here that the molecular concentration of the solutions is as follows:—Phosphates, M/15; borates, M/10; acetates, M/5. The concentration, however, does not materially affect the final equilibrium.

The region of least swelling for the excised sterno-cutaneous is in all solutions from $P_H = 5$ to $P_H = 7$. In the phosphate mixture the weight of the muscle falls to 87·5 per cent. of its initial weight. This region is one in which the muscles are coagulated. They are quite opaque in appearance, and the fibres are contracted. The iso-electric point for the muscle colloids undoubtedly falls within these two values, and it is interesting to note in this connection that the iso-electric points of serum globulin and albumen are also in this neighbourhood. It can be seen from the phosphate curve in fig. 2 that at $P_H = 5$, the muscles are coagulated and shrunken. At $P_H = 4$ on the acetate curve, the hydrogen ion concentration is sufficiently great to cause the muscle to swell to 270 per cent. of its original weight. The rise of the curve on the alkaline side is much more

gradual. It is already noticeable at $P_H = 8$ on the phosphate curve. It has increased very slightly at $P_H = 9$, and also at $P_H = 9$ and $P_H = 10$ on the borate curve. Between $P_H = 10$ and $P_H = 11$ there is a considerable rise, the muscle swelling to 140 per cent. of its initial weight. In the pure caustic soda solution of the same concentration ($P_H = 11 \equiv N/1000$) the muscle swells even more than in the borate solution, and reaches 190 per cent. of its initial weight.

Besides the curves already referred to in figs. 1 and 2 are given the curves obtained by adding hydrochloric acid or caustic soda to an isotonic (0·125 molecular) solution of sodium + potassium + calcium chlorides (Na : K : Ca = 100 : 1 : 1 ; these are the proportions in Ringer's solution). It can be seen that in acid solutions the presence of the salts acts strongly antagonistically to the hydrogen ions. In alkaline solutions the salts at first reduce swelling, though not to a very great extent. Subsequently they seem to increase it. This behaviour may possibly find a parallel in Hardy's observation that acids and salts act antagonistically in their solvent power for globulin, bases and salts act additively (7).

No attempt was made to determine the amount of salt necessary completely to inhibit swelling at any one point on the curve. In view of Hardy and Wood's work on gluten (27), and Loeb and Wasteney's work on the viability of Fundulus (12), one seems on safe ground in assuming that with increasing concentration of acid the amount of salt needed will rise sharply to a maximum and then decline.

It should be noted that all these workers used acid media.

The Time Curves in Acid, Neutral, and Alkaline Media.

So far the curves dealt with in this paper have shown the relationship between the nature of the external medium and the final state of a muscle immersed therein. But the most striking differences in the reactions of an excised muscle towards acid or alkaline media are shown in the curves plotting rate of change of weight in any one medium, *i.e.* the time curves—figs. 3 and 4.

In dilute acid solutions (fig. 3) the curve of swelling is a smooth logarithmic curve rising to a steady maximum, and is similar to that described by Pascheles (15) for the swelling of gelatine, and by Masson (11) for the swelling of cotton fibres. In stronger acid solutions the curve rises smoothly, and then falls again before reaching equilibrium (fig. 3). This rise undoubtedly follows the change of hydrogen ion concentration inside the muscle substance, *i.e.*, as hydrogen ions diffuse into the muscle from the external medium, swelling takes place. This reaches a maximum when the

internal concentration is equivalent to 0·005 normal, and as this concentration is passed a certain amount of shrinkage follows.

In distilled water, or in solutions of neutral salts, or in sugar solutions,

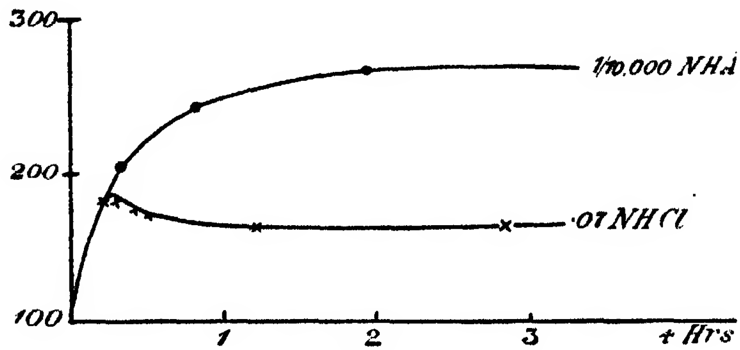

FIG. 3.

Abscissæ = time in hours from beginning of experiment.
Ordinates = weight of muscle expressed in percentage of initial weight.

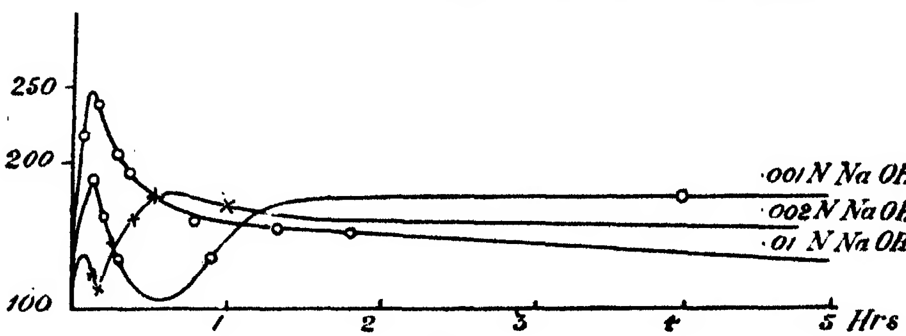

FIG. 4.

Abscissæ = time in hours from beginning of experiment.
Ordinates = weight of muscle expressed in percentage of initial weight.

there may or may not be a preliminary gain in weight, but sooner or later the muscle begins to coagulate, the fibres shorten, and the whole muscle loses weight. This coagulation and loss of weight cannot be prevented by any variation in the tonicity of the solution. The time-weight curves for sugar solutions have already been published (7). The curves for the chlorides of sodium, lithium, potassium, barium, calcium, in isotonic solutions, are given in fig. 5. Following Webster's precedent (26) 0·125 molecular solutions of the monovalent salts, and 0·10 molecular solutions of the divalent salts have been taken as isotonic. They all show the same changes, but the coagulation appears much sooner in the solutions of the divalent metals, while the initial rise in weight is strongly marked in the potassium,

lithium, and ammonium solutions, and fleeting in the calcium and barium solutions. The behaviour of the muscle in sodium solutions is in strong

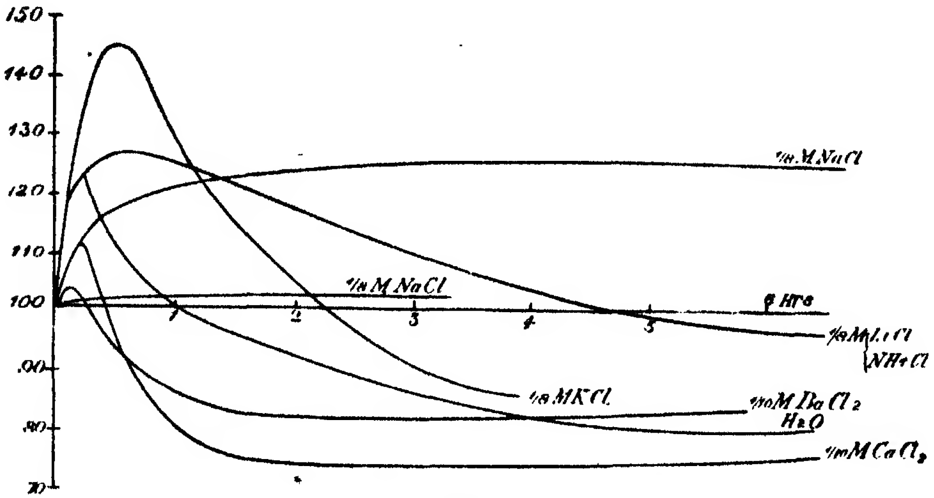

Fig. 5.

Abscissæ = time in hours from beginning of experiment.
Ordinates = weight of muscle expressed in percentage of initial weight.

contrast to its behaviour in the presence of any other single salt, and combined with this is the well-known fact that isotonic sodium solutions are far less toxic for excised muscles than solutions of any other single salt. The behaviour of an excised muscle in 0·125 molecular sodium chloride is mainly determined by its initial state. It may show a rise in weight, or it may remain unchanged for several hours, but in either case the time-weight curve runs for a considerable time parallel to the X axis. Two curves for 0·125 molecular sodium chloride are shown in fig. 5. It might be noted that the Y-axis in fig. 5 is drawn on a different scale to the Y-axis in the other figures. The curves given in fig. 5 are taken from actual experimental results. It should be noted that they represent the mean of measurements which differ from each other somewhat widely.

To turn now to the consideration of the time-weight curves in the case of alkaline solutions—in dilute alkalies, the curve first rises, then falls abruptly for a few minutes, then rises again to a maximum. These curves are shown in fig. 4 for 0·001 normal and 0·002 normal caustic soda.

In dilute alkaline solutions the fall between the two maxima is very clear; as the alkalinity of the solutions is increased, it gets partially obliterated, till it becomes merely a notch in the curve, and, at 0·1 normal, can no longer be distinguished.

The secondary rise of the curve is already quite clear at a concentration $P_H = 8$. In dilute solutions a steady maximum is reached. In strong solutions (as in strong acid solutions) there is a secondary fall, showing slightly at 0·002 normal, and as a violent descent, continuing until the muscle is entirely disintegrated, at 0·1 normal. This secondary effect of alkali on muscle substance makes the numbers given in figs. 1 and 2 for the maximum swelling less reliable than those given for the acid solutions. They are the values obtained in 24 hours, and in strong alkalies (0·07 – 0·1 normal) the muscles undoubtedly lose still more in weight.

In studying the alkali curves, it is noticeable that, with a little experience, it is quite easy to tell what point on the curve the muscle has attained merely by taking note of its appearance. The muscle, when freshly excised, is fairly translucent and yellowish in colour. In the early swelling stages, besides visibly increasing in volume, it turns milky opalescent. As swelling increases, the milkiness gets less and less, and finally a muscle swollen to its full extent is clear and glassy in appearance. A muscle that is losing weight always has its fibres contracted, and often retains a yellow tinge. The history of a muscle hung in alkaline solution can thus be followed by the eye—at first it increases in volume and becomes milky white, then it shortens suddenly and becomes yellow and opaque, finally a glassy appearance begins to show at the edges, and the whole muscle again becomes swollen and clear.

These changes are in intimate relation with the state of the muscle as an electrically charged colloidal system. Evidence has been accumulating for some considerable time that, in the body, the tissue colloids are charged positively, and give an acid reaction. For instance, Fletcher and Hopkins (5) find that working muscles contain between 0·2 and 0·3 per cent. of their total weight of lactic acid, and even in resting muscles they found a persistent minimum of 0·02 per cent. Hardy (8) has shown that, under the influence of an electric current, the colloids of the cell cytoplasm migrate towards the cathode, and it seems a just conclusion, therefore, that they are positively charged. Bollas-Lee, in his 'Microtomist's Vade Mecum' (28), describing the technique of staining *intra vitam* with neutral red, states that tadpoles take up this stain freely, becoming dark red in colour. Now, neutral red is an indicator that is red in solutions with $P_H < 7$, yellow in solutions with $P_H > 8$, and therefore this observation alone is sufficient to show that the tissues are either neutral or acid, but not alkaline. Finally, my own results are most easily explained on the hypothesis that the muscle colloids are an acid, or positively charged system. Consider the muscle *in situ* as a system in equilibrium with an alkaline fluid, the lymph, containing salts.

Removed to an acid-water system, the increase of the hydrogen ion concentration and the removal of the salts both act together to increase swelling.

In a neutral system there are several possibilities. In distilled water, and in hypotonic solutions of sugar, there is an initial swelling of the muscle, due partly to its transference to a medium of lower osmotic pressure and partly to the absence of the normally occurring salts (see further below). In isotonic sodium chloride or Ringer solution the muscle remains for a time of constant weight. The special case of the other neutral salts in isotonic solution will also be considered below. In hypertonic neutral solutions the muscle loses weight from the start. All neutral solutions, however, have this in common, that, sooner or later, a muscle immersed in any one of them becomes coagulated and loses weight, regardless of the tonicity of the solution. This must be due to the loss of all free acid in the muscle by diffusion outwards and to the fact that the iso-electric point of the muscle colloids lies near the neutral point, *i.e.* if it is assumed, as has been done throughout this paper, that the iso-electric point coincides with the point of most complete coagulation, and that the degree of coagulation is measured by the amount of synæresis.

In an alkali-water system the muscle also shows an initial swelling just as in a neutral hypotonic system. But besides water, hydroxyl ions diffuse into the muscle, and so, the acid in the muscle is neutralised. At this point coagulation of the colloid particles and shortening of the fibres occur simultaneously, but, as more hydroxyl ions diffuse into the muscle, the system becomes negatively charged, and swelling again sets in (see the curves in fig. A).

No attempt will be made in this paper to discuss at any length the meaning of the initial swelling in isotonic solutions of potassium, lithium, and ammonium salts. This swelling, under the influence of these salts, can be prevented by injuring the muscle by freezing and thawing (Siebeck, 23), or by saturating the solution used by chloroform. It is therefore possibly connected with the state of the muscle as a living system. Hardy has already brought forward evidence to show that, in living blood, the proteins are present as a single large complex (7, Appendix II), which breaks down under adverse circumstances (such as dialysis), setting free the globulin. Possibly, therefore, in living muscle, these same large complex molecules exist, and the first action of the toxic electrolytes is to cause their breakdown, with an accompanying rise in the osmotic pressure. The coagulating effect of the neutral salts follows later, and is the same in general character as that found in non-living colloid systems, *i.e.* it is a function of the valency of the coagulating ion.

The Relation of Gelatine to Acids, Salts, and Bases.

The curve of maximum swelling of excised muscle in acid or alkaline media is the curve of swelling of some substance in the colloidal state. A search through the literature of colloidal chemistry reveals almost as complete a paucity of systematic data as was found in the more physiological domain of muscular phenomena. Wo. Ostwald (15) has published curves for the swelling of gelatine in acid and alkali. These curves agree with the curves for muscle in showing increased swelling of gelatine with increased concentration of acid and alkali up to a maximum, and afterwards decreased swelling. Ostwald's curves differ from the muscle curves in three important points: firstly, the maxima for both acid and alkali are at a concentration of about 0·025 normal; secondly, the downward slope of the curves beyond these points is very gradual, and finally there is a minimal swelling for acid at 0·005 normal.

The relation of gelatine to acids and salts has also been studied by Proctor (17 and 18). His curve for the swelling of gelatine in dilute hydrochloric acid corresponds remarkably closely with the curve for muscle. The maximum for both curves is in the neighbourhood of 0·005 normal acid, and the curves again rise very steeply to this maximum and slope away sharply beyond it. Proctor explains the swelling of the gelatine on the assumption of the formation of an ionisable salt of the weak diacidic base, gelatine. He finds that this assumption not only explains the fixation of the hydrogen and chlorine ions by the gelatine, but also explains the swelling of the gelatine on a simple osmotic basis. If his theory is valid for gelatine, it must, from the similarity of the two curves, also be valid for muscle, and it leads to the assumption that in a muscle immersed in acid solution, there is chemical interaction between the muscle colloids and the acid, leading to the formation of ionisable salts, which, exerting a definite osmotic pressure against the elastic forces of the muscle, cause swelling. Proctor states that the colloidal cation of his gelatine chloride does not contribute directly to the osmotic pressure. This view, however, is not in harmony with much work that has been done on the direct determination of the osmotic pressure of gelatine gels, and I am not, at the moment, prepared to follow so far.

Finally, Lillie (10) has made an extensive series of experiments by direct measurement on the osmotic pressure of gelatine and egg albumen, and has shown:—

"(1) The osmotic pressure of colloids is unaffected after the addition of non-electrolytes (sucrose, dextrose, glycerine, urea).

"(2) Acid and alkali increase the osmotic pressure of gelatine solutions; in

general these substances affect the osmotic pressure of gelatine solutions in the same manner as they do the rate of swelling of solid gelatine plates immersed in water.

"(3) Addition of salts depresses the osmotic pressure of both colloids; the degree of depression is a function of the nature of both the anion and cation of the salt. It increases in the order, alkali metals $<$ alkali earths (for cations) and Cl $>$ plurivalent anions, phosphates (for anions)."

Muscular Swelling Interpreted as an Osmotic Phenomenon.

I have quoted direct from Lillie's paper because it makes the phenomena of the relations of excised muscles to acids, salts, and bases appear in a clear light. Evidently, acids and bases raise the osmotic pressure of the muscle colloids, and so water flows in; salts depress this osmotic pressure and so less water passes into the muscle. Bechhold (3) and others have stated that sugar does not antagonise the swelling of muscles under the action of acids and bases; and this again falls into line with Lillie's work.

The mechanism by which this osmotic change is brought about follows from Hardy's work on colloidal solutions: acids combine with the colloids in solution to give ionisable complexes which dissociate in such a way that the large colloidal "pseudion" carries a positive charge, and increasing the hydrogen ion increases the degree of dispersity of the colloid particles, and so raises the osmotic pressure of the system (5, 6, etc.). The same holds for the colloids charged negatively by the hydroxyl ion, *i.e.* for colloidal anions. In the presence of neutral salts, the ionisation of the colloid particles of the muscle is suppressed, they lose their charge and are coagulated. This again recalls the salt globulins, which show no movement in an electric field and are brought out of solution by simple dilution. This view harmonises with Proctor's in ascribing to ionisation the controlling factor in the production of the internal osmotic pressure. It differs in considering that the colloidal ions exert a definite quantum to the final result, greater or less according to their degree of dispersity.

It has already been recorded in a previous paper that in hypertonic neutral solutions, the loss in weight of an excised muscle is a linear function of the time, and it was suggested that "the linear form of the curve would imply that the loss is due to a change in the state of the muscle, for if it were merely the establishment of an osmotic balance with a fixed effective mass of solute within the muscle the rate would diminish as the effective concentration within the muscle approached that outside it." This suggestion that the distribution of the common solvent water between the muscle and the external medium depends partly on the direct osmotic pressure of solutes in

the medium, and partly on the configuration of the muscle colloids, is confirmed by researches recorded in this paper which show that the excised muscle is a labile system whose configuration depends on (at least) two variables: (1) the hydrogen ion concentration, (2) the salt concentration.

It should be noticed that in order to explain the swelling and shrinking of muscle on an osmotic basis, there is no need to postulate a semi-permeable membrane at its surface. Moore and Roaf and Webster (14) have urged that the so-called "semi-permeability" of a membrane depends more on the properties of the colloid behind the membrane than on the membrane itself, and in the case of muscles it is easy to show that a muscle which has been coagulated in distilled water (or any other neutral solution) swells immediately on being put into acid or alkaline solution.

So far the properties of excised muscle have been considered purely as a non-living colloidal system. There seems little doubt, however, that the same type of change occurs during life. Ranke (19), Loeb (11), Fletcher (4), and others have shown that the osmotic pressure of muscle rises after fatigue, and the work of Fletcher and Hopkins (5) on the increased lactic acid content of fatigued muscle leaves little doubt that this increased osmotic pressure is due to the increased hydrogen ion concentration. Barcroft and Toyojiro Kato, working on dogs (2), and Cogan, Back, and Towers (1), working on the frog, have shown that fatigued muscles take up water from the body fluids *in situ*, and this must surely again be due to the acid produced.

Ringer in 1883 stated that cardiac muscle will not contract in an acid medium, and that the heart of the frog, perfused with neutral saline until the contractions cease, can be restored by making the solution alkaline. Ringer, who also was aware that the contractions in heart muscle caused the production of acid, considered that the alkalinity of the blood preserved the tissues by preventing accumulation of acid (22). It is suggested below that in the case of the skeletal muscles, whose activity is much more irregular than the cardiac, it is also important to keep the acidity high enough.

Ringer showed for cardiac muscles that loss of contractility does not mean simultaneous death of the tissue. The same is true for the excised skeletal muscles. All the changes described in the foregoing paper are reversible in their early stages. The sterno-cutaneous muscle of the frog, which is swelling in isotonic potassium chloride, can be restored for 15 minutes after loss of contractility, by being placed in isotonic sodium chloride. The same is true for muscles which are losing weight in isotonic (0·1 molecular) calcium chloride. These also can be restored to their normal function for about a

quarter of an hour after they cease to respond to an electric stimulus. As the curves show no break at any moment which might be considered as the death point, it seems justifiable to assume that the osmotic nature of the exchange of water between a muscle and its surrounding medium, which has been shown to depend (1) on the osmotic pressure of the muscle colloids as determined by their state of configuration, (2) on the osmotic pressure of the crystalloids in the external medium, applies equally well to living as to dead muscles.

One point of interest arises in this paper with regard to the living muscle: the working muscle is a positively charged system with a hydrogen ion concentration $P_H < 5$. (This follows from the fact that muscles lose weight slightly in a solution with $P_H = 5$.) The iso-electric point for muscles is between $P_H = 5$ and $P_H = 7$. The reaction of normal blood is $P_H = 7{\cdot}35$ (Walpole). There must, therefore, be an electric potential between the muscles and the body fluids, and since the muscle substance is freely permeable to both hydrogen and hydroxyl ions, it seems possible that one of the results of cell oxidation is that by the constant production of carbonic acid in the muscle, this potential is continually maintained.

Summary.

1. Acids and alkalies both cause swelling in excised muscle. The degree of swelling is not directly proportional to the concentration of acid or alkali in the surrounding fluid but has a maximum at 0·005 normal for hydrochloric acid and for caustic soda. Alkalies first coagulate and then redissolve the muscle substance.

2. The chlorides of the alkali and alkaline earth metals all ultimately coagulate the protoplasm of an excised muscle in isotonic solutions. The bivalent cations show this effect much more rapidly than the monovalent. Distilled water and sugar solutions also coagulate excised muscles.

3. The iso-electric point for muscle is between $P_H = 5$ and $P_H = 7$.

4. It is suggested that the swelling and shrinking of muscles both in the body and out is an osmotic phenomenon, and that the configuration of the colloids of the muscle substance is the chief determining factor which fixes the degree of swelling. Lillie's demonstration that acids and alkalies raise the osmotic pressure of gelatine, while the neutral salts lower it, is in harmony with this view.

5. The osmotic phenomena of muscle can be fully explained without assuming the presence of a semi-permeable membrane round the muscle fibres.

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A Theory of Colour Vision.

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Notes on the Genus Toxoplasma, *with a Description of Three New Species.*

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[Plates 9 and 10.]

The genus *Toxoplasma* has not yet any definite systematic position. By some, the organisms which bear this name are regarded as being allied to the Leishmania, and by others to the yeasts. This uncertainty must exist so long as we do not know the complete life-history of these organisms; but, since any addition of new facts concerning them may help towards the acquisition of this knowledge, it has been thought worth while to record the finding of three new varieties, and to describe them.

The organisms were first seen by Splendore (1), and were described by him in July, 1908. He found them in the blood of a rabbit in Brazil, and the name *Toxoplasma* was given to them by Nicolle and Manceaux (2), who found them in a gondi in Tunis, shortly after Splendore's discovery, and described them in October, 1908. Since then, others have been found in the dog by Mello in Italy and by Yakimoff in Germany, in the mole by Prowazek in Japan, and in the pigeon by Carini in Brazil.

It seems probable, so far as our knowledge of these organisms extends at present, that they are more nearly related to the Hæmogregarines than to any other of the Hæmosporidia; they have no micronucleus, and therefore cannot belong to the Leishmania, as Nicolle and Manceaux thought, and the absence of a distinct capsule, and the fact that no budding process has been observed, should prevent them from being regarded as yeasts, as has been suggested.

The Toxoplasmas may occur free in the blood, but are generally found in the large mononuclear leucocytes; their distribution in the body of the affected animal is often peculiar. They give rise to very marked wasting and to considerable blood destruction, almost as marked as in the Babesia infections.

The Toxoplasmas are organisms of either a crescentic or bi-convex shape, sometimes with pointed and sometimes with rounded ends; occasionally, and under pressure, they may become nearly round. There is no capsule or definite membrane to be seen around them. The protoplasm is very fine

and delicate, and sometimes contains vacuoles. They are, on this account, difficult to fix satisfactorily. I have found that fixation by the vapour of iodine dissolved in chloroform, as described by me in 'Roy. Soc. Proc.,' B, vol. 86, p. 389, gave the best results. The nucleus consists, in what may be regarded as a normal parasite, of a round dot or karyosome, which, before division of the cell, lengthens and becomes rod-shaped and then dumb-bell-shaped. Rarely, a ring form of nucleus may be found, as in Plate 9, fig. 6.

In many of the organisms the nucleus is either broken up into granules, or the cell gets filled with granules which take chromatin stains (hæmatoxylin, fuchsin, thionin, have been those used), so that they are indistinguishable from the nucleus. These granules may be the so-called "infective granules," such as have been described by the late Major W. B. Fry and Captain Ranken, V.C. (3), in Trypanosomes, which the late Prof. Minchin (4) acknowledged in the last paper written by him; for the amount of ordinary division seen does not seem to correspond with the enormous number of parasites found in individual leucocytes. They have none of them the reaction, with iodine fixation, of the reserve food granules seen in Trypanosomes.

The varieties of *Toxoplasma* I have found in a fossa, a fruit pigeon, and a Say's snake, showed no motility when examined fresh, even on the warm stage, neither did I observe any changes of shape.

As regards size, the organisms vary too much in the same animal for very approximate measurements to be of much use. In the fossa they were the smallest, and varied from $2\,\mu$ to $8\,\mu$ in straight length from end to end, and from $1{\cdot}4\,\mu$ to $2{\cdot}5\,\mu$ in breadth at middle. In the fruit pigeon the length varied from $3\,\mu$ to $8\,\mu$, and the breadth from $2\,\mu$ to $5\,\mu$. Those found in Say's snake were larger, and varied from $7\,\mu$ to $10\,\mu$ in length, and from $3\,\mu$ to $6\,\mu$ in breadth. These measurements were made on fresh, unfixed organisms.

In all these cases the Toxoplasmas were found free in the blood, but in very small numbers; they were generally found in the large mononuclear leucocytes from the affected organs, often occurring in enormous numbers. They were found principally in the lungs and pleural effusion and in the bone-marrow in the fossa; in the lungs and exudation from the lungs in the fruit pigeon, and in the liver in Say's snake. None were found in the bone-marrow of the bird or snake. The infected leucocytes show but little alteration in the early stages of the infection, when they contain only one or two parasites, but as these multiply the leucocyte enlarges enormously, and the protoplasm becomes extremely thin and frothy in texture, and there is always a marked hyperchromatosis of the nucleus; the cell eventually

breaks up. The parasites are sometimes found in the nucleus itself; of this an example is shown in Plate 9, fig. 2. There is a tendency for these leucocytes to mass themselves together, but no true giant-cells are formed. Very few parasites were found in the endothelial cells of these three animals; a few were found in the omentum of the fossa and in the mesentery of the fruit pigeon. This is quite contrary to the experience of Miss H. L. M. Pixell (5), who found cells containing large numbers of the parasites from these situations.

The multiplication of the Toxoplasmas is effected ordinarily by longitudinal division. The nucleus first enlarges, then becomes rod-shaped, and later of dumb-bell shape, and eventually the daughter nuclei are formed, the cell having already begun to divide. I have seen no example of transverse division in these cases. In the bone-marrow of the fossa several round cysts were found in enlarged leucocytes, which suggested schizogony (Plate 9, fig. 3), and these are somewhat like the schizont in the Coccidia when it is about to divide up into merozoits; and in the Say's snake (Plate 10, fig. 9) there is a structure which is apparently a later stage, showing the formation of merozoits. No flagellated forms have been seen.

Many attempts at cultivation in various media were made, but none were successful.

The *Toxoplasma* has a very wide geographical as well as zoological distribution. The three cases here recorded came respectively from Madagascar, the Aru Islands, and Mexico, and those previously described by various observers were found in Brazil, Tunis, Italy, Japan, and Germany.

The varieties to be described were found in the course of the *post-mortem* examination of the animals which have died in the Zoological Gardens, and the following paragraphs will indicate the distribution of the parasites and the points of difference between them :—

I. *Fossa*, Cryptoprocta ferox, *from Madagascar.* Plate 9, figs. 1–3.

The animal was very wasted. Both pleural, peritoneal, and pericardial cavities contained a quantity of blood-stained fluid. The lungs and kidneys were very congested, and there was a layer of lymph on the under-surface of the diaphragm. The blood was extremely anæmic, and contained many poikilocytes and nucleated erythrocytes. A few Toxoplasmas were found in the blood; many were found in the blood from the lung, and in the pleural and peritoneal exudation, and in the bone-marrow. Few were found free; nearly all were contained in the large mononuclear leucocytes, often a great number, as many as 36, in a single leucocyte. The leucocytes were very much enlarged, and their protoplasm was extremely thin and delicate, many

being ruptured in the preparation of the film, even with the greatest care. The nucleus of the leucocyte invariably showed signs of hyperchromatosis, often very marked. The nucleus of the parasite was often broken up into granules, or chromidia, but many showed the single dot form with a clear area around. Sometimes the *Toxoplasma* was found in the nucleus itself (Plate 9, fig. 2). Schizonts were found in the bone-marrow in various stages up to the apparent breaking up into merozoits (Plate 9, fig. 3). Occasionally the parasites were found in the polynuclear cells in the bone-marrow: this was possibly a phagocytic process, as the shapes of the ingested parasites were much altered.

II. *Blue-tailed Fruit Pigeon,* Carpophaga concinna, *from the Aru Islands.* Plate 9, figs. 4–6; Plate 10, fig. 10.

Died in an emaciated condition. The lungs were very congested, and contained a large quantity of exudation. There was some bloody fluid in the body cavity. Very few parasites were found in the blood, but large numbers were present in the blood and exudation from the lungs. Some were found free, but they were mostly contained in the large mononuclear leucocytes. These cells had undergone more destruction than was the case in the fossa, and the blood was extremely anæmic. The nucleus of the *Toxoplasma* was generally single and definite, and was not broken up into granules. A few were found in the bone-marrow, but none showing definite schizogony. The drawing, reproduced in Plate 10, fig. 10, was made to scale from an unfixed preparation, just tinted with 1–2000 methylene blue in 0·8 per cent. salt solution. At 1, there are ordinary forms of the *Toxoplasma;* at 2, a form with the nucleus in form of chromidia or granules; at 3, a large mononuclear leucocyte containing several parasites, some quite differentiated; and at 4, possibly an early stage of schizogony.

III. *Say's Snake,* Coluber melanoleucus, *from Mexico.* Plate 10, figs. 7–9.

The snake was very wasted, and its blood, which was very anæmic, contained a few hæmogregarines. The lung was pneumonic and full of exudation; the liver was small and pale. Toxoplasmas were found in small numbers in the exudation from the lung, and in enormous numbers in the liver; a few single ones in the blood.

The above is all that it has been possible to find in this material. A careful watch is being kept for new cases, from which it is hoped to obtain material which will enable the knowledge of this curious parasite to be carried further.

I am much indebted to Mr. A. T. Watson, the laboratory assistant at the Zoological Gardens, for very intelligent help in the preparation of the specimens, and to Dr. A. Norman for the photographs of a difficult object.

[*Note added May* 1, 1916.—Since the above was written I have found Toxoplasmas in another bird, a Pied Bush Chat (*Pratincola caprata*) from India. They were found in the blood and exudation from the lungs, which were inflamed and œdematous, in the mononuclear leucocytes as before. Further early stages of schizogony were found in the large endothelial cells in the lungs.]

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EXPLANATION OF PLATES 9 AND 10.

Figs. 1–3, from Fossa.

The preparations were photographed under a magnification of 1000 diameters.

They were fixed wet in the vapour of iodine dissolved in chloroform, and were stained with Giemsa's stain, made alkaline, and followed by acetone and xylol.

Fig. 1.—Mononuclear leucocyte in blood from lung, showing enlargement of leucocyte, and the delicate, frothy protoplasm, with hyperchromatosis of the nucleus. The leucocyte contains two parasites, one of ordinary shape just to left of nucleus, and a larger one with undefined extremities at the upper part of the cell.

Fig. 2.—Two mononuclear leucocytes from the peritoneal exudation, both showing hyperchromatosis of the nuclei. The cells are filled with parasites, and in the cell on the left the nuclei of the parasites are broken up into granules. In this cell a parasite is seen in the nucleus.

Fig. 3.—A large mononuclear leucocyte from the bone-marrow, with considerable hyperchromatosis of the nucleus, containing a mass of parasites, apparently in process of schizogony, and of breaking up into merozoits. These were found in many stages.

Figs. 4–6 and 10, from Fruit Pigeon.

Fig. 4 fixed by the method of Carnoy-Lebrun, and stained with Weigert's hæmatoxylin. Figs. 5 and 6 fixed and stained as Figs. 1–3. Photographed × 1000.

FIG. 4.—From exudation from lung, showing three free toxoplasmas to the right, and to the left a mononuclear leucocyte, with a hyperchromatic nucleus, containing parasites, two apparently in late stage of division.

FIG. 5.—From same exudation, showing three free toxoplasmas at upper part, a large mononuclear leucocyte containing one parasite on right, and a similar cell containing several on the left.

FIG. 6.—From blood from lung, showing a large mononuclear leucocyte containing several toxoplasmas. Below the hyperchromatic nucleus can be seen two parasites with ring-shaped nuclei.

FIG. 10.—Drawing made from fresh unfixed specimen of exudation from Fruit Pigeon's lung. 1. Ordinary toxoplasmas. 2. Form with nucleus in form of granules. 3. Large mononuclear leucocyte containing parasites. 4. Probably an early stage of schizogony.

FIGS. 7–9, FROM SAY'S SNAKE.

Fig. 7 fixed in vapour of iodine dissolved in chloroform and stained with alkaline Giemsa's stain. Fig. 8 fixed by method of Carnoy-Lebrun and stained with Weigert's hæmatoxylin. Fig. 9 fixed as Fig. 8 and stained with alkaline Giemsa's stain. Photographed × 1000.

FIG. 7.—From liver, showing a fusion of three large mononuclear cells containing a number of parasites. It will be noticed that the toxoplasmas lie in vacuoles, and that they are larger than those in either the mammal or the bird.

FIG. 8.—From liver, showing one very distended mononuclear leucocyte, with nucleus about to break up, and with very delicate frothy protoplasm, apparently about to give way, containing 14 parasites, each lying in a distinct vacuole.

FIG. 9.—A section from the liver, showing several free toxoplasmata in lower part, and above what is probably a later stage of schizogony than that shown in Plate 9, fig 3, as the shape of the parasites is more defined.

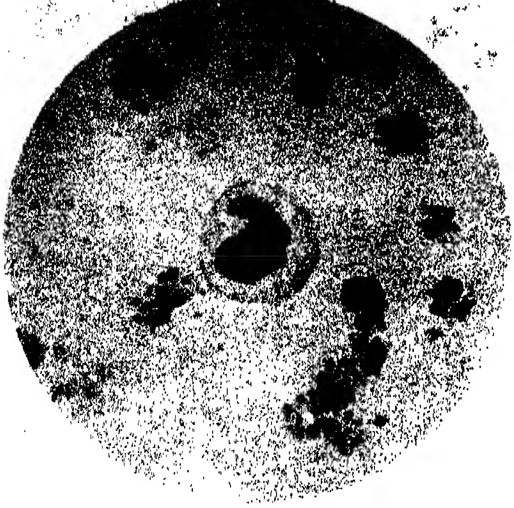

FIG. 1.

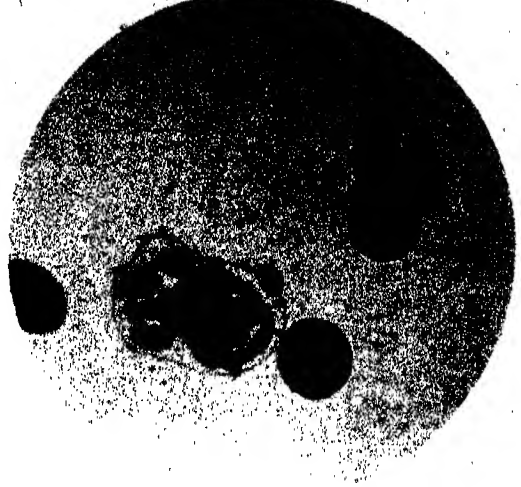

FIG. 4.

FIG. 2.

FIG. 5.

FIG. 3.

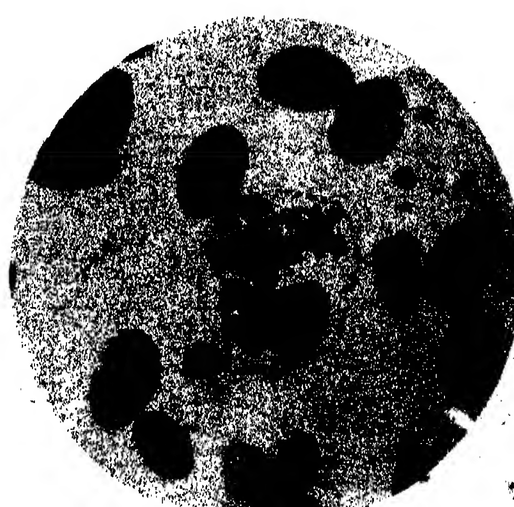

FIG. 6.


FIG. 7.

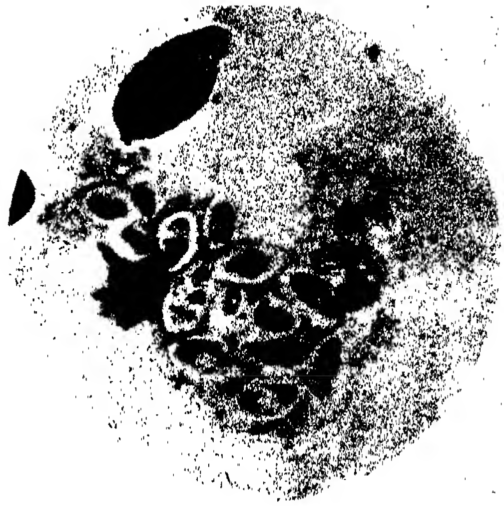
FIG. 8.


FIG. 9.

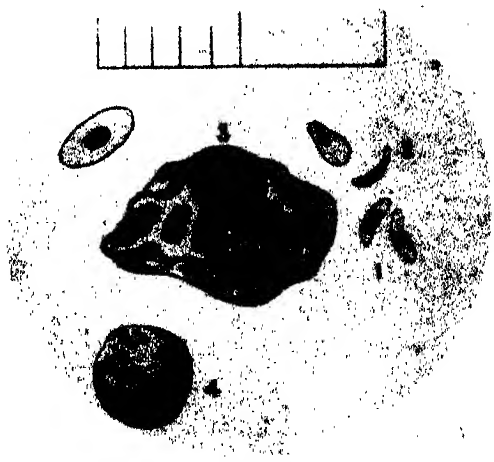
FIG. 10.

Further Observations on Protozoa in Relation to Soil Bacteria.

By T. Goodey, D.Sc., Protozoologist, Research Laboratory in Agricultural Zoology, University of Birmingham.

(Communicated by Prof. F. W. Gamble, F.R.S. Received March 16, 1916.)

CONTENTS.

Introduction.

The work recorded in the present paper is a continuation and an extension of my earlier work on the inoculation of protozoa into soil, an account of which has already been published in these Proceedings.* I pointed out in that paper (p. 455) that the introduction of large numbers of bacteria into the soil along with the added protozoa must be a source of disturbance to the bacterial flora, and by reason of this the experiments could not be considered as showing a clear issue between the added protozoa and the soil bacteria. The investigations dealt with in the present paper represent an attempt to eliminate this source of experimental error, my aim being primarily to inoculate a partially sterilised soil with protozoa freed from bacteria.

The work was begun some months before Russell's paper† replying to my previous communication appeared, so that the experiments described here are open to much of the criticism levelled at the earlier work. However, as I have obtained certain very interesting results which are worthy of record, and as I may not be able to continue the investigation much longer, it seems desirable to publish an account of the work, and give the results obtained. My best thanks are due to Prof. F. W. Gamble for his helpful criticisms and suggestions throughout the course of the work, and especially during the preparation of this paper.

* 'Roy. Soc. Proc.,' B, vol. 88, p. 437 (1915).
† 'Roy. Soc. Proc.,' B, vol. 89, p. 76 (1915).

Methods.

The soil used was a fibrous loam obtained from stacked turf. It was friable, and worked easily, containing about 18 per cent. of water by weight. Lots of 400 grm. were put up in quart bottles previously sterilised and plugged with cotton-wool. In order to partially sterilise the soil it was treated with 2 per cent. of toluene, which was allowed to act for two days, after which the soil was spread out in order to allow the antiseptic to evaporate. By inoculating some of the soil treated in this way on to nutrient agar, it was found that amœbæ and flagellates developed after a few days' incubation. The soil was therefore heated in a steamer regulated at 65° C. for 2 hours, in the hope of killing off all the remaining protozoa. At the end of this treatment bacterial counts by the gelatine plate method were made, and the moisture content of the soil determined. More agar plates were inoculated with small quantities of the soil to test for the presence of protozoa, and after about 10 days' incubation a few amœbæ and flagellates were found on the surface of the medium, thus showing that the heating had not eliminated all the protozoa. However, no further attempt was made to eradicate these remaining forms except in the case referred to on p. 301, and the inoculation of the desired protozoa into the soil samples was proceeded with.

In order to obtain a large number of protozoa free from bacteria I devised the following method, which, in brief, consisted in driving by means of an electric current free-swimming ciliates and other protozoan forms from a medium in which bacteria abounded into a similar medium free from bacteria. For this purpose a glass trough supported in a wooden frame was constructed. It measured about 7 inches in length by 4 inches in width, and was divided transversely into two equal parts by an upright glass partition which did not quite reach to the height of the two sides, and thus allowed of free communication between the two halves over the top of it. The trough, supported on the stand of a dissecting microscope, is shown in fig. 1. In using the apparatus I proceeded as follows. A mixed culture of ciliated protozoa from soil was made in 1-per-cent. hay-infusion, the following organisms being present:—*Colpoda cucullus*, *Col. maupasii*, *Col. steinii*, *Gonostomum affine*, *Urostyla* sp., besides a large number of amœbæ and flagellates. The surface layers of the culture containing a vast number of organisms were skimmed off and put on one side, whilst the bulk of the culture was filtered through a sterile Berkfeld filter so as to free it from bacteria. The mixed culture of protozoa and bacteria was poured into one side of the trough to the requisite height and into the other half was poured

filtered culture liquid. A ridge of plasticine was placed along the top of the glass partition in order to prevent the contents of each half mixing

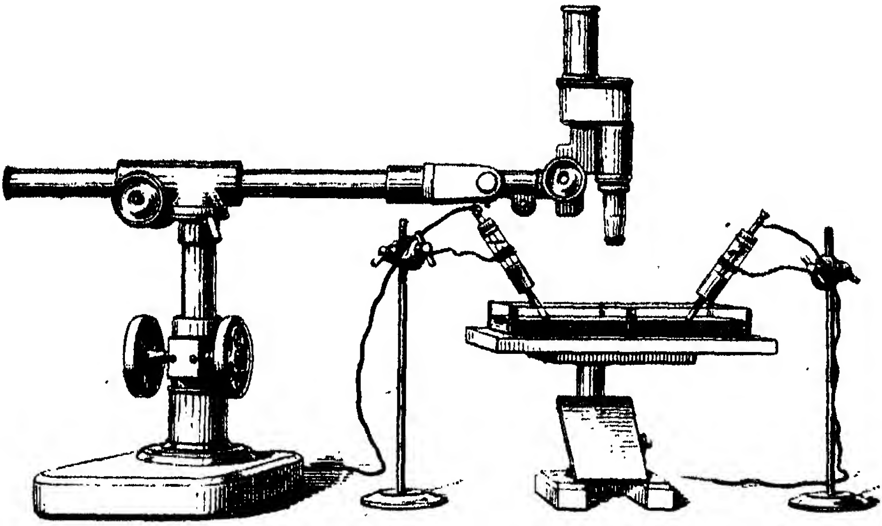

FIG. 1.—Apparatus used for separating protozoa from bacteria; for description, see text.

together. When sufficient filtered liquid had been added the plasticine was gently removed and there was a slight flow from the filtered liquid into the side containing the protozoa. An electric current was now passed through the liquid in the trough by means of non-polarisable electrodes. The latter was constructed in exactly the same way as those described and figured in an earlier paper.* The current used was obtained from the 220 volts electric-light circuit and served the purposes of the experiment excellently. A large dissecting microscope was used for observing the protozoa in the trough. It was arranged that the current should flow through the liquid in the trough from the mixed culture to the filtered liquid, and when the current was switched on the ciliated protozoa immediately began to swim with the current over the glass partition into the filtered liquid, where they soon became so numerous that they could be seen with the naked eye as motile white particles. After allowing the current to flow for about five minutes the vast majority of the ciliates had been driven over in the desired manner. The current was then switched off and the plasticine ridge replaced on top of the glass partition. The liquid originally containing the mixed culture of ciliates, etc., was then siphoned off and the trough was carefully wiped out with a wad of cotton-wool, which

* Goodey, 'Roy. Soc. Proc.,' B, vol. 84, p. 172 (1911).

soaked up the last traces of liquid. Fresh filtered liquid was then poured into this side of the trough until it rose above the level of the top of the glass partition. The plasticine was again removed, allowing a flow of filtered liquid into the side containing the protozoa. The electric current was reversed and again switched on, this time causing the protozoa to swim back into the fresh filtered liquid, in which a large number were obtained. By this means the protozoa were washed in one lot of bacteria-free liquid and finally secured in a comparatively clean condition. Bacterial counts showed that the original mixed culture contained about 36 million bacteria per cubic centimetre, whilst the liquid in which the protozoa were finally obtained contained only 300,000 bacteria per cubic centimetre. Thus a very considerable reduction in the numbers of bacteria was effected, although it was not possible to rid the protozoa entirely of bacteria : a result scarcely to be expected seeing that large free-swimming ciliates might easily carry over bacteria on the surface of their bodies. The requisite quantity of this liquid containing "bacteria-free" protozoa was then taken and used for moistening one of the samples of partially sterilised soil.

Another sample of treated soil was inoculated with a large number of amœba cysts free from bacteria, obtained by the following method of Cropper and Drew.* A single cyst of a small limax amœba, obtained originally from soil, was picked off from the surface of an agar plate by means of a capillary pipette. The cyst was lying in an area free from bacteria, having moved out to the clean agar in advance of the growing bacteria. This cyst was next transferred to a fresh agar plate and an emulsion of *Bacillus fluorescens non-liquefaciens* in sterile distilled water was added. After incubating at 20° C. for a few days the plate was examined, and it was found that the single cyst had given rise to a large number of amœbæ. Two or three more fresh agar plates were then inoculated from this culture of amœbæ and *B. fl. non-liq.*, and were left in the incubator until the amœbæ had all encysted. A 2-per-cent. solution of hydrochloric acid (2 per cent. of the ordinary pure reagent) was then added to the cultures and allowed to act for two days. It was then poured off and the surface of the cultures washed with several changes of sterile distilled water so as to get rid of all traces of acid. This treatment killed off the bacteria but left the amœbæ cysts uninjured, as was proved by making sub-cultures of the cysts on to fresh agar plates. On these, bacteria failed to appear, but when a suspension of *B. fl. non-liq.* was mixed with the cysts and the mixture inoculated on to nutrient agar, the amœbæ excysted and readily multiplied. Quantities of the cysts were obtained by gently scraping the surface of the agar, and

* 'Researches into Induced Cell-reproduction in Amœbæ,' p. 83. London, 1914.

these were made into a suspension in sterile distilled water, a measured volume of which was used for moistening one of the soil samples. This particular lot of soil, together with one to act as a control, had been submitted to a further treatment by heating to 75–80° C. for 5 or 6 hours with a view to getting rid of the amœbæ and flagellates which had survived the previous heating to 65° C.

In order to estimate the numbers of protozoa in soil, cultures were made in 1 per cent. hay-infusion and on agar plates, as described in my previous communication (p. 442).* It has been found impracticable to weigh smaller quantities of soil than 0·0002 grm., and for estimating protozoa further than this point a dilution method was employed in which 0·001 grm. of soil is shaken up with 10 c.c. of sterile normal salt solution, and then measured quantities of the suspension are transferred to the surface of nutrient agar plates, three plates being used for each dilution; 1 c.c. of such a suspension is equivalent to 0·0001 gram of soil, and so on. I may add that this method has yielded satisfactory results. The agar-plate method has been found the more serviceable, being especially good for amœbæ, whilst the hay-infusion is better for ciliated protozoa, as illustrated by the fact that whilst from the Toluened *plus* ciliates (T+C) soil I could obtain no *Colpoda cucullus* by the agar-plate method, by the use of hay-infusion this organism appeared in culture regularly down to a dilution of 1 in 1000. Bacterial counts were made periodically by the gelatine-plate method.

The complete set of soils as finally put up so as to include various controls consisted of the following:—Untreated (U), Toluened *plus* sterile distilled water (T+W), Toluened *plus* 5 per cent. untreated soil (T+5 per cent. U), Toluened *plus* ciliates in filtered hay-infusion (T+C), Toluened *plus* mixed culture of protozoa and bacteria in hay-infusion (T+M), Toluened *plus* filtered culture, hay-infusion without protozoa and bacteria (T+F), Toluened and twice heated *plus* amœbæ (T+A), Toluened and twice heated (TH_2).

Results.

The results of the periodical bacterial counts are given in the Tables on p. 302, and are represented graphically in figs. 2, 4 and 5.

* 'Roy. Soc. Proc.,' B, vol. 88 (1915).

Bacteria in Millions per Gramme.

	At start.	After 21 days.	After 43 days.	After 78 days.	After 104 days.	After 154 days.	After 190 days.	After 223 days.	After 293 days.	After 323 days.
U	20	25	24	lost	25	21	16	21	23	
T + W	3	130	150	100	lost	85	91	124	lost	106
T + 5 per cent. U......	—	162	96	91	lost	66	103	120	50	
T + F	—	320	323	144	136	67	63	107	56	
T + C	—	603	396	154	lost	126	87	121	105	
T + M	—	595	335	209	166	131	87	111	56	

Bacteria in Millions per Gramme.

	After 23 days.	After 58 days.	After 84 days.	After 134 days.	After 170 days.	After 208 days.	After 273 days.
T + A	60	151	60	53	54	58	53

Bacteria in Millions per Gramme.

	After 27 days.	After 58 days.	After 103 days.	After 139 days.	After 172 days.	After 242 days.
TH_2	64	82	62	64	56	63

1. *Inoculation of Treated with 5 per Cent. Untreated Soil.*

I propose to deal first with the results obtained from the (T + 5 per cent. U) soil, as these are the most important.

Fig. 2 shows the curves representing the bacterial counts for the untreated, the (T + W) and the (T + 5 per cent. U) soils. It will be seen that the (T + 5 per cent. U) curve shows a sharp rise at 21 days, reaching a much

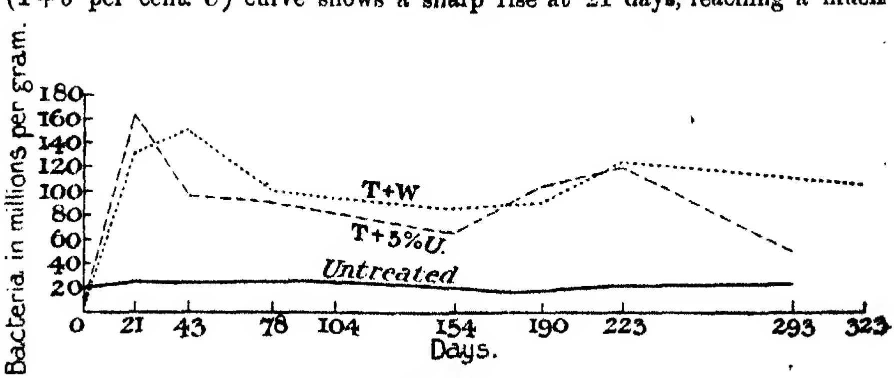

Fig. 2.

higher point than that of the (T+W) soil. This is followed by a marked drop at 43 days, whilst the (T+W) curve shows a continued rise up to this time, though later on it shows a drop at 78 days. From this point onwards until the 154th day the (T+5 per cent. U) curve keeps at a lower level than the (T+W) curve. Both curves rise to a fairly high level at the 223rd day, following a re-moistening of the soils, whilst at the 293rd day the (T+5 per cent. U) curve has dropped to a comparatively low level again, at which time the bacterial count for the (T+W) soil was unfortunately lost owing to the liquefaction of the gelatine plates. A later count, however, at 323 days showed that the numbers had kept high. The sharp rise at 21 days, and the subsequent marked drop at 43 days in the (T+5 per cent. U) curve are characteristic features of curves of treated *plus* small quantities of untreated soil, as Russell and Hutchinson have shown in many experiments. These two investigators have also attributed the fall in the numbers of bacteria to the depressing effect of the activity of the protozoa added in the untreated soil.

It will be convenient at this point to deal with the protozoal counts for the soils under consideration, and in the accompanying Table the approximate numbers of protozoa per gramme of soil are set out.

	Untreated. Nos. per gramme.	T+W. Nos. per gramme.		T+5 per cent. U. Nos. per gramme.		
	At start.	At start.	After 320 days.	At start.	After 43 days.	After 210 days.
Ciliates—						
Colpoda steinii	50	—	—	2 or 3	100	
Gonostomum affine	? a few	–	—	? a few	50	
Rhizopods—						
Amœba limax	10,000	100	200	600	30,000	30,000
Amœba terricola	? a few	—	—	? a few	200	1,000
Gephyramœba delicatula	? a few	—	—	? a few	200	1,000
Flagellates, sps. ?	10,000	1000	—	1500	10,000	?

The following protozoa occurred so irregularly in the untreated soil cultures that it was impossible to form an approximate estimate of their numbers; only a few of each species, however, appeared to be present per gramme of soil:—*Colpoda cucullus*, *Col. maupasii*, *Gonostomum affine*, *Chilodon* sp.?, *Balantiophorus* sp.?, *Amœba terricola*, *Leptomyxa flabellata*,* *Gephyramœba delicatula*,* and *Chlamydophrys* sp.?.

In the (T+5 per cent. U) soil the numbers at 43 days are very striking, especially those for *Amœba limax*, and show that within six weeks from the

* Goodey, T., "A Preliminary Communication on three new Proteomyxan Rhizopods from Soil," 'Arch. f. Protistenkunde,' vol. 35, p. 89 (1914).

beginning of the experiment there has been an increase in the numbers of this organism from about 600 to 30,000 per gramme, whilst for *Amœba terricola* and *Gephyramœba delicatula* there has been a multiplication from a few per gramme to about 200 per gramme of each species.

The flagellates also show evidence of marked activity, having increased from 1500 to 10,000 per gramme. The two species of ciliates have also increased considerably in numbers. This multiplication of protozoa has gone hand in hand with the depression in the bacterial numbers at 43 days and the only conclusion, I think, that can be drawn from this is that the bacterial decrease in numbers is the direct effect of the protozoal increase in numbers. This result definitely supports the hypothesis of Russell and Hutchinson and constitutes the first record of its kind in the course of the investigations on this problem. The results of the protozoal counts seem to suggest that the chief part in the depressing action of the protozoa on the bacterial numbers at 43 days must be ascribed to the *Amœba limax*, which attained the high figure of 30,000 per gramme. It is interesting to note also that the later count at the 210th day shows no increase in the number of *Amœba limax* over the 30,000 per gramme reached at 43 days, whereas the *Amœba terricola* and *Gephyramœba delicatula* have each increased from 200 to 1000 per gramme.

From this it would appear possible that a particular soil may only be capable of supporting a certain maximal number of one species of amœba, which in the case of this soil is about 30,000 per gramme of the *Amœba limax* in question. This is, however, merely a tentative suggestion.

The Table shows that the Toluened plus sterile distilled water soil (T + W) had initially about 100 *Amœba limax* and 1000 flagellates per gramme, whilst counts made at 320 days showed about 200 *Amœba limax*, and no flagellates, the latter failing to appear in the cultures. This indicates that there has been practically no activity and multiplication on the part of the amœba in this soil. In the (T + 5 per cent. U) soil, however, there has been marked activity and multiplication of the protozoa, and from this it would appear that a treated soil does not afford a suitable medium for protozoal activity and that in order to render it suitable a small quantity of untreated soil must be added to it.

2. *Confirmatory Experiment.*

In view of the important character of the results just described, another experiment was started in order if possible to obtain confirmation of them. A quantity of soil from the same source as that used in the earlier experiment was taken and partially sterilised by heat. For this purpose 800 grm. were taken and put up in thin test-tubes and then put into a steamer regulated at 72° C. and left there for five hours in the hope of killing off all forms of protozoa.

The soil was given this treatment in preference to toluening followed by heating as used in the earlier experiment because it was found that the latter treatment did not eradicate all the amœbæ and flagellates. Agar plates inoculated with small quantities of the heated soil showed no amœbæ or flagellates after a week's incubation and from this it was concluded that all the protozoa had been killed off. The 800 grm. of heated soil were divided into two equal portions of 400 grm. and to one of these 5 per cent. of untreated soil was added and thoroughly mixed with it. Both lots were bottled in the usual way, the mouths of the bottles being furnished with cotton-wool plugs. The water content of all three lots, Untreated (U), Heated (H), and Heated *plus* 5 per cent. untreated (H + 5 per cent. U), was 20 per cent. by weight.

The protozoal counts giving the approximate numbers of organisms per gramme of soil are set out in the accompanying Table.

	Untreated. Nos. per gramme.	Heated (H). Nos. per gramme.	(H + 5 per cent. U.) Nos. per gramme.			
	At start.	At start.	At start.	After 19 days.	After 44 days.	After 92 days.
Ciliates—						
Colpoda steinii	100	None	5			
Rhizopods—						
Amœba limax..............	3000	,,	150	1000	5000	30,000
Amœba terricola	50	,,	5	100	200	2,000
Gephyramœba delicatula	20	,,	1	10	20	500
Chlamydophrys sp. ?	20	,,	1	—	—	20
Flagellates, sps. ?	1000	,,	50			

The following ciliates cropped up so irregularly in the cultures of the untreated soil that no real estimate could be formed of their numbers; only a few species were present per gramme of soil:—*Colpoda cucullus*, *Col. maupasii*, *Enchelys* sp. ?, *Chilodon* sp. ?, *Gonostomum affine*, and *Pleurotricha* sp. ?.

The periodical bacterial counts are set out in the Table on p. 306, and their graphic representation is shown in fig. 3.

Considering now the curves for the bacterial counts, we notice that the usual rise in the (H + 5 per cent. U) soil, reaching its culmination at 50 days, is followed by a drop at 85 days, whilst the (H) curve during the same period continues to rise. In the protozoal counts we find that the rhizopoids have multiplied enormously in numbers during the same period, the *Amœba limax* reaching 30,000 per gramme 7 days after the numbers of bacteria have become depressed. The other organisms mentioned in the Table also show evidence of marked activity; but I wish to emphasise

Bacteria in Millions per Gramme.

	At start.	After 17 days.	After 50 days.	After 85 days.	After 126 days.	After 162 days.
U	30	16	20	12	12	12
H	2·7	65	75	120	96	70
H + 5 per cent. U	—	86	138	95	78	58

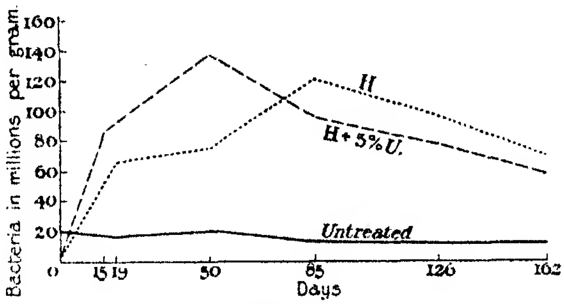

Fig. 3.

especially, at this point, the figures for the *Amœba limax*. These are the same as those for *Amœba limax* in the earlier experiment, where 30,000 were present at 43 days, when the drop occurred in the numbers of bacteria. The result of the second experiment fully confirms that of the first, and points conclusively to the fact that the activity of the protozoa has had a depressing effect upon the numbers of bacteria. A further point brought out is that the limiting action of protozoa is chiefly operative through the activity of a small *Amœba limax*, and that for this particular soil, at any rate, we can say that the necessary number of this amœba to produce the effect is about 30,000 per gramme.

In the earlier experiment the depressing effect occurred at 43 days, and in the confirmatory experiment at 85 days, which is 42 days longer. It is not difficult, however, to suggest an explanation of this. If we refer to the number of *Amœba limax* present at the beginning of the experiment we shall see that in the first case (T + 5 per cent. U) there were about 600 per gramme, whilst in the second case (H + 5 per cent. U) there were about 150 per gramme. Thus there was a much greater initial number in the one case than in the other, and consequently a shorter time would be necessary for them to multiply and reach the figure at which they would have the depressing effect upon bacterial numbers. Moreover, in the treated portion of the (T + 5 per cent. U) soil all the amœba had not been killed off as in the treated portion of the (H + 5 per cent. U), and these remaining forms,

about 100 per gramme, would no doubt multiply alongside the added protozoa, having a suitable medium in which to grow, and thus contribute towards the more speedy attainment of the 30,000 in this soil than in the other, where all the protozoa were eradicated.

3. *Mass Inoculations of Protozoa.*

Turning now to a consideration of the curves for the other soils. Toluened *plus* ciliates in hay-infusion (T+C), Toluened *plus* mixed culture of protozoa and bacteria in hay-infusion (T+M), and Toluened *plus* hay-infusion filtered through a Berkfeld filter (T+F), which are represented in fig. 4

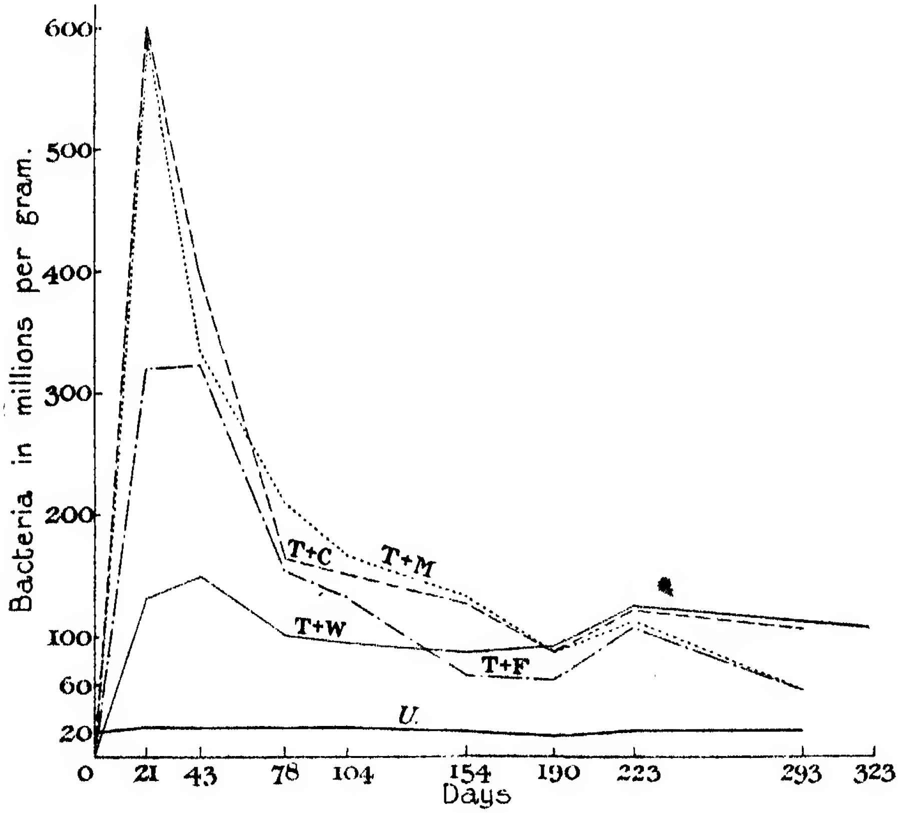

Fig. 4.

along with the curves of Toluened *plus* sterile distilled water (T+W) and the untreated (U), we notice a marked difference from the curves already discussed.

The (T+C) and (T+M) each show a remarkably high bacterial content at 21 days, followed by an equally sharp drop at 43 and 78 days, after which the fall in numbers is more gradual. Only at 190 days do they reach a

point below the level of the (T+W) curve. The (T+F) curve reaches a high level at 23 days, but not so high as that attained by the (T+C) and (T+M) curves, and this is maintained at 78 days. After this it drops rapidly, and at 154 days is well below the level of the (T+W) curve, a position it maintains onwards to the end. The rise shown by all the curves at 243 days is explicable, owing to the re-moistening of the soils.

These curves are very similar to some of those dealt with in my previous communication,* as in figs. 1, 2, and 6.

The very high bacterial numbers attained in the (T+C), (T+M), and (T+F) soils are to be accounted for, I believe, by the same explanation as that put forward for the similar high counts in the earlier work, viz., that by the addition of hay-infusion to the treated soil the bacteria of the soil are given large quantities of food upon which they thrive and multiply enormously, and that as these supplies of food become used up the numbers of bacteria fall.

The curves for the (T+C) and (T+M) soils are very much alike, and one can say without hesitation that the same influences have been at work in each case. The interesting fact also emerges from the consideration of these two curves, namely that the elimination of the vast majority of the bacteria by the electrical method described above has not had the effect of decreasing the bacterial content of the (T+C) soil as might have been expected; on the contrary, the (T+C) count at 21 days is slightly higher than the (T+M) count.

The protozoal counts for these soils are shown in the following Table in approximate numbers per gramme of soil:—

	T + C. Nos. per gramme.		T + M. Nos. per gramme.		T + F. Nos. per gramme.	
	At start.	Towards end.	At start.	Towards end.	At start.	Towards end.
Ciliates—						
Colpoda cucullus	1,000	500	1,000	1,000		
Col. maupasii	30	? a few	30	? a few		
Pleurotricha sp. ?	50	? a few	100	? a few		
Rhizopods—						
Amœba limax	3,000	20,000	20,000	20,000	1,000	1,000
Amœba terricola	—	—	2,000	2,000		
Flagellates, sp. ?	10,000	10,000	5,000	5,000	1,000	1,000

The folowing ciliates occurred in cultures of the (T+M) soil so irregularly that no approximate estimate of their numbers could be formed; only a few of each species were present per gramme of soil: *Colpoda steinii*, *Chilodon* sp. ? *Enchelys* sp. ? and *Epistylis* sp. ?

* 'Roy. Soc. Proc.,' B, vol. 88 (1915).

The drop in bacterial numbers in all three inoculated soils (T + C), (T + M), and (T + F) is not, I think, to be attributed to protozoal activity. The only curve which reaches well below the level of the (T + W) curve is that for (T + F). Now in this sample there were, as the Table shows, about 1000 *Amœba limax* and 1000 flagellates per gramme, at the beginning of the experiment, whilst in the other two soils there were much greater numbers of protozoa. Moreover, towards the end of the experiment protozoal counts showed that there had been no increase in numbers of protozoa in the (T + F) soil, and from this it seems reasonable to infer that there had been no activity of the amœbæ and flagellates. Consequently the drop in the numbers of bacteria must be attributed to some other influence than protozoal action. This statement may appear to invalidate the previous results, but reference should be made to p. 311 under "Discussion," where an explanation is advanced which covers this and the similar cases from the (T + C) and (T + M) soils. In the same way later protozoal counts for (T + C) and (T + M) soils showed signs of increase in the numbers of protozoa in only one case, and that is for *Amœba limax* in (T + C), where the figure for this organism has risen from 3000 to 20,000 per gramme.

The ciliate *cucullus* in (T + C) has actually decreased from 1000 to 500 per gramme, and in like manner the other ciliates are not so numerous as at the beginning of the experiment.

4. *Mass Inoculations of Bacteria-free Amœba Cysts.*

The last curves to be considered are those of the Toluened and twice-heated soil *plus* amœbæ free from bacteria (T + A), and the Toluened and twice-heated soil (TH_2), which are shown in fig. 5.

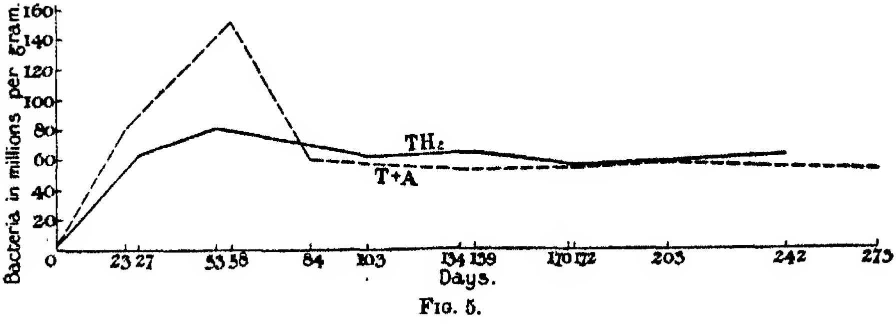

Fig. 5.

The method by which the amœba cysts used in this experiment were prepared has been described above (p. 300). The protozoal counts made

at the beginning of the experiment showed approximately 30,000 amœbæ per gramme, whilst those made towards the end of the experiment show the same number per gramme, thus indicating that there has been no activity and multiplication of the organisms added. For this reason I do not interpret the fall in the curve of the bacterial numbers in (T + A) as due to the action of amœbæ. How then is the sharp rise and the subsequent drop in the curve to be accounted for? The only explanation I can furnish, and I think that it meets the situation, is that the nutrient agar on which the amœbæ had been growing, and of which small quantities were unavoidably added along with the encysted amœbæ, supplied a suitable extra food substance for the bacteria of the soil, and that they multiplied on this, and when it was exhausted their numbers fell again. It thus comes into line with the soils inoculated with hay-infusion, where the extra food supplied gave rise to an enormous increase in bacterial numbers.

The 30,000 amœbæ per gramme were added in an encysted condition, and there is no evidence that they ever excysted and became active, probably for the same reason that the protozoa in the other treated and inoculated soils failed to become active and to multiply, *i.e.*, because of the unsuitability of the treated soil as a medium for active trophic existence.

Discussion.

The failure of the inoculated protozoa in the (T + C) and (T + M) soils and of the residual protozoa in the (T + F) soil to multiply, except in the one case of the *Amœba limax* in (T + C), is interesting, and is probably open to the same explanation as that advanced above for the similar phenomena in the (T + W) and (T + A) soils, viz., the treated soil, for some obscure reason, does not afford a suitable medium for protozoal activity. This of itself is probably a sufficient explanation, but two other points are worthy of consideration in the case of these soils. Very high numbers of bacteria were present early in the experiment, and these may have tended to check protozoal activity, for Cunningham* has shown that the presence of large numbers of bacteria checks the development of protozoa in a medium. It is also possible where a soil receives a mass inoculation of protozoa sufficient to give large numbers of protozoa per gramme that this in itself may tend to inhibit their further increase in numbers.

A comparison of the curves from the mass inoculation experiments of the present and the previous investigation reveals a remarkable similarity in

* 'Journ. Agric. Sci.,' vol. 7, Part I (1915).

their shape. In all cases we get an early rise in bacterial numbers to a very high level, followed by a rapid drop. This drop shows the coming into action of some factor limiting bacterial activity, and the explanation which I have advanced is that this is a nutrient rather than a protozoal factor. The food added in the form of hay-infusion serves as a suitable medium for the attainment of high bacterial numbers in the soil, and on the exhaustion of this food the bacteria naturally fall in numbers.

The protozoal counts in the present investigation show that there is little or no multiplication of the protozoa added by mass inoculation, whereas the counts in the previous work did not allow of such an interpretation, for the initial and the later counts were not made by strictly comparable methods, as was pointed out in the earlier paper (p. 442). I have no doubt now, however, in view of the results from the mass inoculations in the present investigation, that there was no activity and multiplication of the protozoa added by mass inoculation in the earlier investigation.

The drop in the curves from the experiments in the addition of 5 per cent. of untreated to treated soil in the present investigation shows the effect of protozoal activity on bacterial numbers, and the curves must therefore be interpreted as showing a protozoal rather than a nutrient limiting factor. In the same way the curves for the (T + 5 per cent. U) and (1846 + 10 per cent. 1870) soil of the previous investigation, which showed evidence of some factor checking bacterial increase, should probably be interpreted as showing the effect of a protozoal limiting factor. The evidence at hand at the time of writing the earlier paper did not warrant this conclusion, but it was fully recognised that some factor had been introduced with the untreated soil which tended to depress the numbers of bacteria. As a consequence, however, of the results obtained in the present research, by the addition of untreated to treated soil, it seems reasonable to bring the earlier results into line with those of the present investigation, though one cannot show the marked correlation between protozoal increase and bacterial decrease in numbers in the earlier work as one can in the later.

Three facts emerge clearly from the present investigation:—

1. Where a treated soil has comparatively few protozoa added to it along with a small amount of untreated soil, these protozoa can and do multiply in the mixture, and after a time exert a depressing effect upon bacterial numbers.

2. When large numbers of protozoa are added in cultures to a treated soil, whether with or without bacteria, little or no multiplication of the protozoa takes place, and there is no evidence of their exerting a limiting action on bacterial activity.

3. The residual protozoa of a treated soil fail to increase in numbers, even though the conditions are favourable to great bacterial activity. 2 and 3 show that the treated soil alone is an unsuitable medium for active trophic existence of protozoa.

Another point calls for some comment. Russell has claimed,* on the strength of Martin and Lewin's results,† that the *culture* fauna is distinct from the *trophic* fauna of a soil, and has criticised the results in my previous communication by suggesting that there is nothing to show that in the introduction of mass protozoa cultures the real trophic fauna was reintroduced into the soil. I agree that the criticism is probably justified. At the same time, however, I would suggest that too much emphasis should not be laid on the distinction between the *trophic* and the *culture* fauna, for the results which I have recorded from the (T + 5 per cent. U) and (H + 5 per cent. U) soils show that at least two or three species of amœbæ, viz., *Amœba limax*, *Amœba terricola*, and *Gephyramœba delicatula*, are capable of leading an active existence and of multiplying in the soil, and must therefore have constituted the trophic fauna, or an important part of it, during the course of the experiment. These are all forms which occur readily in culture; in fact, it is by the agar-plate method that I have been able to demonstrate their presence and active multiplication in the soil.

A word also is perhaps necessary here on the numerical estimation of soil protozoa, especially in view of the remarks of Martin and Lewin on this subject in their last joint paper.‡ The results given for the (T + 5 per cent. U) and the (H + 5 per cent. U) soils show that the agar-plate and the hay-infusion methods are practicable for giving rough approximations of the numbers of soil protozoa. The methods as employed in this piece of research are probably more reliable than the dilution method, in which soil is shaken up with a liquid followed by the transference of measured quantities of the soil suspension to nutrient media, for I have inoculated both agar-plates and hay-infusion with actual weighed quantities of soil, from 1 grm. down to 0·0002 grm., and hence have eliminated certain of the experimental errors incident to the use of the dilution method, which has only been used for obtaining quantities of soil smaller than 0·0002 grm.

As methods they undoubtedly leave much to be desired, for there are so many uncontrollable factors entailed in their use. At the same time they are the only methods at present available, and one is justified, I think, in

* 'Roy. Soc. Proc.,' B, vol. 89, p. 82 (1915).

† 'Phil. Trans.,' vol. 205, pp. 79–94 (1914); and 'Journ. Agric. Sci.,' vol. 7, pp. 106–119 (1915).

‡ 'Journ. Agric. Sci.,' vol. 7, p. 109 (1915).

drawing conclusions even of first-rate importance from the results obtained by their use, as has been done in the present investigation.

SUMMARY.

1. A method is described whereby protozoa were successfully separated from large numbers of bacteria and used for mass inoculation of a treated soil. No positive evidence was obtained by the use of this method on the power of the protozoa so treated and added to soil to function as a limiting factor to bacterial activity.

2. Further negative evidence on protozoal activity similar to that adduced in an earlier communication is given by the use of mass inoculation of protozoa into soil, whether the organisms are added in mixed culture with bacteria or after separation from bacteria electrically, as in the case of ciliates, or chemically, as in the case of encysted amœbæ. The curves for these experiments are interpreted as showing the action of a nutrient limiting factor. Suggestions are put forward which may furnish an explanation of the failure of the protozoa so inoculated to function.

3. Positive evidence in support of Russell and Hutchinson's hypothesis of the limiting action of protozoa on bacterial increase is brought forward as the result of experiments in which 5 per cent. of untreated soil is added to a partially sterilised soil. The drop in bacterial numbers in mixtures of soils of this sort after an initial rise is well known from Russell and Hutchinson's results, but the further fact is revealed in the present investigations that concomitantly with this bacterial decrease in numbers there is increase in protozoal numbers. Another point brought out in the two experiments of this class is that a certain number of a small amœba of the *limax* group is present in the soil at the time when the drop in bacterial numbers occurs. This number is approximately 30,000 per gramme in each case, and the periodic protozoal counts show that there has been active multiplication of this amœba leading up to the attainment of this number, in one soil from an initial 600 amœbæ per gramme and in the other from an initial 150 per gramme. In the former case the 30,000 per gramme was reached in 43 days, at which time the drop in bacterial numbers occurred, whereas in the confirmatory experiment, which had an initial 150 amœbæ per gramme, the 30,000 per gramme were present after 92 days, at which time also the bacteria had dropped in numbers. There can be no doubt that the two phenomena of protozoal increase and bacterial decrease, as shown in the two experiments, are related as cause and effect. There is evidence also from these experiments of the activity and multiplication of two other forms of rhizopods, viz., *Amœba terricola* a large thick pellicled form, and *Gephyramœba*

delicatula a member of the Proteomyxa, and also of the flagellates. The (T + 5 per cent. U) soil also showed evidence of the activity and multiplication of certain large ciliates, whilst the (H + 5 per cent. U) did not.

Conclusions.

1. Protozoa, especially amœbæ of the *limax* group and other larger forms, can lead an active existence and multiply in soil and exert a depressing effect on bacterial numbers.

2. It is probable that for a given soil a certain point must be reached in protozoal numbers before the depression in bacterial numbers is caused. In the soil under investigation this number appears to be approximately 30,000 *Amœba limax* per gramme.

3. It appears to be necessary to add the protozoa to a treated soil in a small quantity of untreated soil to ensure their having a suitable medium in which to grow and multiply. Under these conditions it is shown that they can increase in numbers and depress the numbers of bacteria.

4. It does not appear to be possible to carry out mass inoculations of protozoa into treated soil in such a way that they come into action and limit bacterial activity, and the explanation advanced to account for this failure is that the treated soil alone affords an unsuitable medium for the active trophic existence of protozoa.

On the Occurrence of Gelatinous Spicules, and their Mode of Origin, in a New Genus of Siliceous Sponges.

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[PLATE 11.]

The siliceous microscleres, or flesh-spicules, of the tetraxonid sponges have long been regarded by those who have studied them as amongst the most beautiful and at the same time most inexplicable structural phenomena met with in the organic world. Their exquisite symmetry, their great diversity in form in different genera and species, and their remarkable constancy in details of shape within the limits of the same species, taken in conjunction with the fact that it is impossible to account for this shape by reference to any function that they may perform in the vital economy of the sponge, constitute a problem of no little interest to the philosophical biologist.

These spicules are generally stated to be composed of hydrated silica, or opal. So far as has been known hitherto, they are perfectly transparent, hard and brittle, and are unaffected by prolonged boiling with strong acids. They are universally believed to be intracellular in origin, and on several occasions have been figured within nucleated mother-cells. The evidence on this point is, however, rather scanty and by no means conclusive, and though several observers have studied the mature form of these spicules in great detail, singularly little is actually known about their mode of origin.

The chief object of the present communication is to describe an entirely new type of spicule, differing, not so much in form as in chemical composition, from any previously known, the study of which, it is hoped, may throw considerable light on the nature and origin of siliceous microscleres in general.

The most striking and novel feature about the spicules in question is that, although still composed, so far as can be ascertained, of colloidal silica, they are gelatinous, contracting greatly in alcohol and swelling up again on addition of water. I first observed them in Australia many years ago, when engaged in cataloguing the great collection of Victorian sponges made by the late Mr. Bracebridge Wilson. They were found in only a single specimen, and their nature was so problematical and the sponge in which they occurred so ill-characterised in other respects that I set it aside as "indeterminable at present," and did not include it in my published catalogue.

Recently, while investigating and reporting upon a large collection of sponges from the Indian Ocean, made by the "Sealark" Expedition under the leadership of Prof. Stanley Gardiner, I have come across three more specimens containing similar spicules, and have been led to make a detailed examination of these enigmatical bodies. The "Sealark" specimens belong to a perfectly distinct species, but probably related not distantly to the Australian sponge. I propose for the reception of the latter the new genus *Collosclerophora*. A diagnosis of this genus and a brief description of the type species, *Collosclerophora arenacea*, are given at the end of the present paper. I reserve an account of the Indian Ocean sponge for my report on the "Sealark" collection.

Unfortunately the amount of material at my disposal for the investigation of the jelly-spicules of *Collosclerophora arenacea* was extremely small. There remained in my possession only a rough, unstained Canada balsam mount, consisting of a single thick, hand-cut section prepared more than twenty years ago.

This preparation showed the jelly-spicules or "collosclercs," as I propose to term them, with great distinctness and in very large numbers, scattered through the soft tissues. They varied considerably in shape, as shown in Plate 11, fig. 1, some being sausage-shaped, others boomerang-shaped, and others again kidney-shaped, but always with a more or less distinct notch or indentation on one side. The length of these bodies, measured in a straight line from end to end, varied from about 0·02 to 0·03 mm. Although they had perfectly sharp outlines, they were somewhat less bright in appearance than ordinary microscleres, and it was at once evident that there was, apart altogether from their shape, something peculiar about them.

In order further to investigate the nature of the collosclercs it was necessary to remove the section from the slide on which it was mounted. This was effected by soaking in xylol, the Canada balsam being thus completely extracted. Part of the section was remounted in balsam, and the remainder transferred to alcohol.

After soaking in xylol the collosclercs almost completely disappeared from view, so that in the fragments remounted in xylol balsam they were barely recognisable, though the ordinary spicules were of course brilliant, there being a great difference in refractive index between the two. I found them little, if any, more distinct in chloroform balsam, and it would appear as if very prolonged soaking in balsam were necessary to render them as distinct as they were in the original preparation. They are not really soluble either in xylol or chloroform.

The original section unfortunately consisted chiefly of sand-grains, which

had to be picked out one by one with needles in order to make preparations suitable for more minute examination, but sufficient of the sponge-tissue remained to enable me to make the following observations.

When mounted in a drop of absolute alcohol the collosclercs are perfectly distinct and may sometimes be seen to be enclosed each in a thin-walled vesicle (fig. 2). When a drop of water is run in under the cover-glass of such a preparation the colloscleres suddenly swell up to several times their previous volume and become extremely transparent (fig. 2*a*; fig. 3, *b*, *c*). The swelling takes place chiefly on the convex side, which becomes more strongly convex, while the line of demarcation between it and the surrounding water becomes almost invisible. The contour of the concave side, on the other hand, is quite bright and distinct, and usually distinctly double. I conclude from this that the substance of which the collosclere is composed is denser and less absorbent of water on the concave than on the convex side. I also conclude that in life these spicules, if spicules they can be termed, exist in the sponge in the swollen, gelatinous condition, and that the contracted state first observed is due simply to the withdrawal of water by means of alcohol. It seems remarkable that they should retain their power of absorbing water and swelling up even after more than twenty years in Canada balsam.

In a very strong cold solution of caustic potash the colloscleres, already swollen in water, swell up further and then dissolve.

When a 5 per cent. solution of hydrochloric acid is run in under the cover-glass they swell up and become invisible in the surrounding tissues, but there is no effervescence and I do not think they really dissolve. At any rate, the contour of the concave surface may remain distinctly visible even after prolonged action of strong hydrochloric acid (fig. 3, *b'*), and a preparation stained with paracarmine and mounted in Canada balsam after treatment with dilute hydrochloric acid shows at least one collosclere quite distinctly.

The colloscleres stain quite readily with paracarmine, but with a solution of iodine in potassium iodide I obtained only negative results. When examined with the polariscope they exhibit no optical activity either when contracted in alcohol or when swollen in water. From these observations I think it may be concluded with a reasonable degree of certainty that the colloscleres are composed of colloidal silica containing a much higher percentage of water than the ordinary siliceous spicules of the same sponge.

Teased preparations, stained with paracarmine and mounted in Canada balsam after removal of the sand-grains, throw a great deal of light on the origin of the colloscleres. They are found to be associated in the mesoglœa with large spherical cells, which, for reasons which will appear directly,

I regard as their mother-cells or scleroblasts (silicoblasts). These scleroblasts occur in groups, sometimes so closely packed together as to become polygonal from mutual pressure (fig. 4), sometimes more loosely arranged (figs. 5–8). Each one is about 0·02 mm. in diameter and provided with a very thin cell-membrane. When darkly stained (figs. 5 and 6) the whole cell appears rather coarsely granular and no nucleus is visible. When more lightly stained (fig. 4) it is seen that the cell is vesicular and contains a small nucleus, apparently suspended in the middle by threads of cytoplasm, while the highly refringent granules lie against the inner surface of the cell-membrane. Except for the presence of these granules the scleroblasts agree very closely with the well-known form of sponge-tissue termed by Sollas cystenchyme.

Amongst the scleroblasts lie the collosclercs; each is commonly enclosed in an oval, thin-walled vesicle very much larger than itself (figs. 5, 6, 8), though it seems probable that in life it may have completely filled the vesicle. The wall of the vesicle stains very distinctly and in much the same way as does the collosclere itself.

It is by no means easy to determine the exact relations between the collosclercs, their vesicles, and the scleroblasts. In teased preparations the vesicles, with their contained collosclercs, sometimes appear quite separate and isolated, but there is, I think, no question of the vesicle itself being the wall of a mother-cell. Not infrequently one vesicle can clearly be seen attached sideways to a single scleroblast, as shown in figs. 9 and 10, and in such cases it is hard to resist the conviction that the vesicle, or, at any rate, its contents, is in some way or other the product of the scleroblast. In such cases the collosclere usually lies at the side of the vesicle remote from the scleroblast, with its concave surface turned towards the latter.

In other cases a vesicle may appear to be attached end-on to a scleroblast, as shown in fig. 8, but I am not satisfied that the association in such cases is not accidental, and that the scleroblasts that really belong to the vesicles in question have not been removed in the course of preparation. In fig. 11 a vesicle is shown which has quite evidently been torn partially away from a scleroblast, but whether its own or not it is again impossible to say.

The strongest evidence that the collosclercs really are secreted by the cells which I have ventured, perhaps somewhat prematurely, to call the scleroblasts, is as follows. A large proportion of these cells are found to exhibit a well-defined rounded knob, attached to the outer surface of the cell membrane at one pole, as shown in fig. 3, *a*, and fig. 7. In fig. 7 the four cells represented do not seem to have been disarranged in the course of preparation, and each one shows the characteristic knob, all the knobs, curiously enough, pointing in approximately the same direction. These

knobs offer a striking contrast to the cells themselves in their hyaline, non-granular character. They stain fairly darkly with paracarmine and appear to be entirely devoid of structure. They are attached to the scleroblasts by broad bases and vary considerably in size, progressive stages of growth being represented in figs. 12–16. They evidently represent an extracellular secretion of the scleroblasts, possibly derived from the minute refractive granules which line the inner surface of the cell-membrane. The largest knobs are of just about the same size as the contracted collosclercs, but, unlike the latter, they do not lie in hollow vesicles and do not swell up on addition of water. This fact seems, at first sight, to negative the view that the knobs are really the collosclercs in process of secretion, but I think the apparent discrepancy may possibly be explained as follows.

We have already seen that the collosclercs always exhibit an indentation or notch on one side, which we may conveniently term the hilum. This may be taken to represent the original attachment of the colloscler to the scleroblast, an attachment which is strongly suggested by the appearance represented in fig. 9. We have also seen that, on the addition of water, the contracted colloscler swells up chiefly on the convex side, the concave surface, which is supposed to lie next to the scleroblast, being apparently formed of much denser silica. We are therefore, I think, justified in assuming, at any rate provisionally, that the secretion, when first discharged from the scleroblast, is in a concentrated condition, and that it only acquires the property of absorbing water and swelling up after the lapse of a longer or shorter interval. It seems not unlikely that the swelling up may be coincident with its complete separation from the scleroblast.

Probably, in life, as I have already suggested, the swollen colloscler completely fills the vesicle in which it lies, and the wall of the vesicle may be regarded either as a concentration of the mesoglœa due to the pressure of the colloscler, or as a precipitation membrane formed at the surface of contact between the gelatinous colloscler on the one hand and the gelatinous mesoglœa on the other, the former consisting of colloidal silica and the latter presumably of an albuminoid character.*

It appears, then, that the collosclercs are gelatinous spicules of colloidal silica, formed by special scleroblasts or mother-cells, but as extracellular and not intracellular secretions. They are undoubtedly a normal constituent of the sponges in which they have been found, and, as I have already pointed out, they occur in two perfectly distinct, though related species. In the Indian Ocean species they are much smaller than in the Australian, and are associated with smaller scleroblasts. I have not traced their development

* Probably both contain small quantities of mineral salts.

in this species, but I have been able to demonstrate with the greatest ease that they swell up on the addition of water just as they do in the Australian sponge.

In the Indian Ocean species they are also associated with large numbers of minute palmate isochelæ of the usual Clathria type. Indeed, until I discovered their property of absorbing water and swelling up, I had no doubt that the collosclerес in this sponge were merely modifications of these isochelæ, with the space between the shaft and palms filled up with silica, a view which was strongly supported by the occurrence of what appear to be intermediate forms.

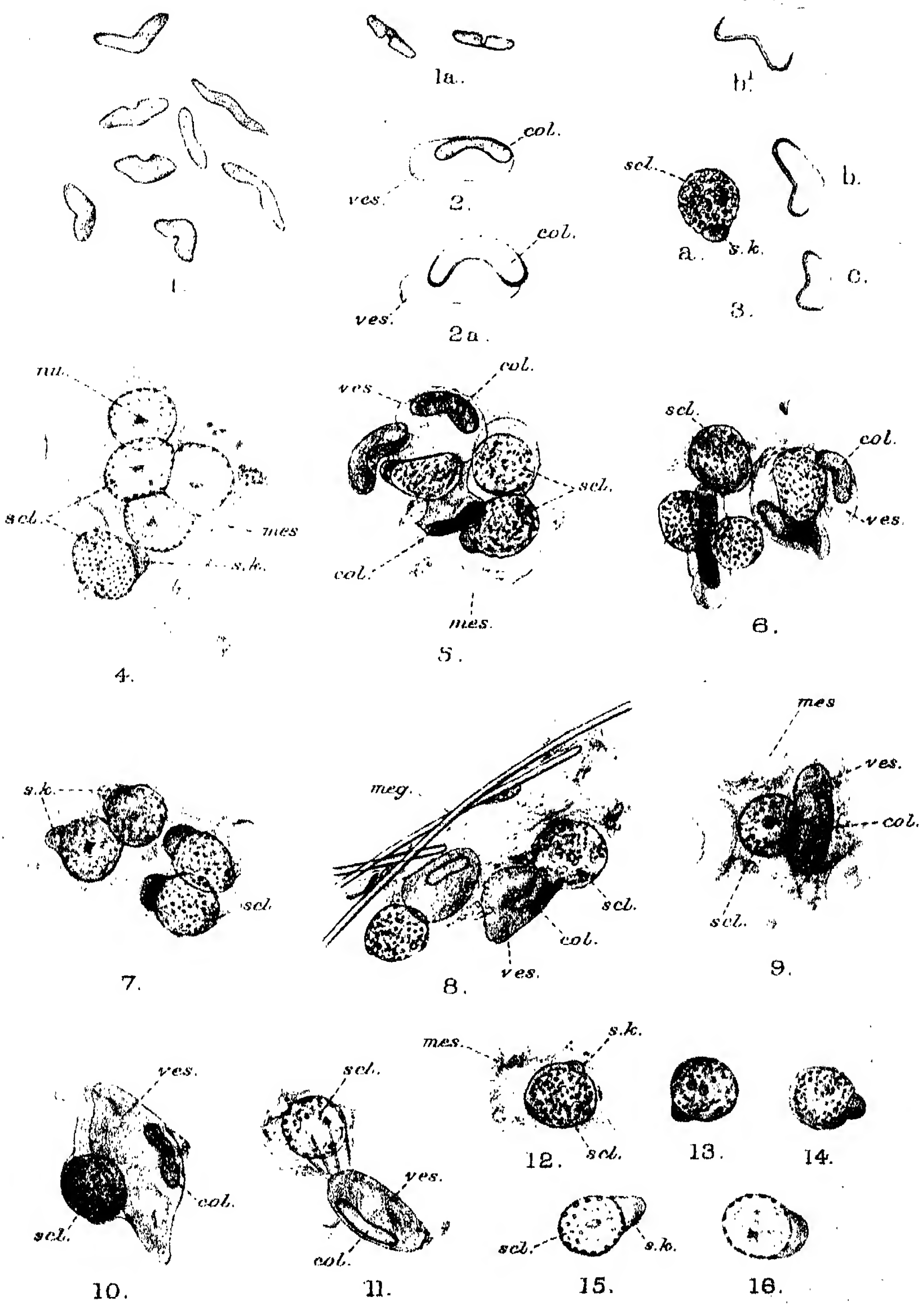
In *Collosclerophora arenacea* I have seen only one or two isochelæ and it is doubtful if such spicules any longer form a normal constituent of the very degenerate spiculation, though here again one occasionally sees what look like intermediate forms between collosclerеs and isochelæ (fig. 1*a*).

In view of the generally accepted ideas as to the intracellular origin, not only of chelæ, but of microscleres in general, it would no doubt be premature to insist upon the homology of the collosclerеs with isochelæ. Such ideas, however, are not based upon a very firm foundation. It seems not impossible that what have hitherto been taken to be the nuclei of mother-cells are really, at any rate in the case of the chelæ, entire scleroblasts adhering to the spicules, while the supposed membrane of the mother-cell is the wall of an extracellular spicule-vesicle. Further investigations into the origin of siliceous microscleres are urgently called for, and until these have been carried out it is inadvisable to propound any general theory on the subject. The occurrence of gelatinous spicules is in itself, however, such a remarkable and unique phenomenon that it seems desirable to place it on record without further delay.

Genus Collosclerophora n. gen.

Diagnosis.—Tetraxonid sponges with gelatinous microscleres (collosclerеs). The normal skeleton is almost entirely replaced by sand-grains. The megascleres are slender strongyla.

The type-species of the genus (*Collosclerophora arenacea*) is a typical sand-sponge with much reduced spiculation, and were that species alone available for study it would hardly be possible to form a definite conclusion as to its proper position in the tetraxonid series. Fortunately the Indian Ocean species, which will be described in my Report on the Sponges of the "Sealark" Expedition, still preserves a full complement of both mega- and microscleres, which enables me to refer it without hesitation to the



Arthur Dendy del. Huth, London.

Ectyoninæ, and to infer that the Australian species probably belongs to the same sub-family, though perhaps generically distinct.

Collosclerophora arenacea n. sp.

Sponge massive, sessile, solid, with evenly rounded convex upper surface showing parallel sandy tracts, separated by intervening areas with minutely reticulate dermal membrane. Vents small, scattered in intervening areas. Texture incompressible, friable, intensely sandy, the sand being arranged in a lamino-reticulate fashion.

Megascleres.—Slender strongyla, straight or nearly so, measuring about 0·22 by 0·0025 mm., smooth and with evenly rounded ends. These spicules are very numerous, occurring chiefly in loose wisps radiating towards the surface, where they form sparse surface-brushes. There is no other dermal skeleton.

Microscleres (figs. 1, 1*a*).—Collosclercs of varying form. When contracted, always with an indentation or notch on one side; sausage-shaped, boomerang-shaped and kidney-shaped. These spicules swell up and become gelatinous on addition of water (figs. 2*a*; 3, *b*, *c*.).

The single specimen was dredged by Mr. J. Bracebridge Wilson in the summer of 1888–9 at Station 1, near Port Phillip Heads, and is entered as R.N. 923 in my manuscript catalogue, from which the above details as to external characters are taken.

DESCRIPTION OF PLATE 11.

Collosclerophora arenacea n. gen. et sp.

Fig. 1.—Collosclercs in the contracted state, as seen in an old Canada balsam preparation, unstained. ×460.

Fig. 1*a*.—Two contracted collosclercs from the same preparation, approaching isochelæ in form. ×460.

Fig. 2.—A collosclere enclosed in its vesicle, separated in absolute alcohol. ×480.

Fig. 2*a*.—The same collosclere after swelling up in water. ×480.

Fig. 3.—A scleroblast (*a*) and two collosclercs (*b* and *c*) examined in water, unstained. The collosclercs have swollen but the secretion-knob on the scleroblast has not; *b'*, the same collosclere as represented in *b*, after running in a drop of fuming hydrochloric-acid from the edge of the cover-glass. ×480.

Fig. 4.—A group of scleroblasts surrounded by mesoglœa; from a teased preparation, very lightly stained with paracarmine and mounted in Canada balsam. The four upper cells show the nucleus very distinctly; the lowest one is focussed on the cell-membrane and shows a secretion-knob. ×480.

Fig. 5.—A group of scleroblasts in the mesoglœa, with associated collosclercs and vesicles. From a teased preparation stained with paracarmine and mounted in Canada balsam. ×480.

Fig. 6.—Another group of scleroblasts with associated collosclercs and vesicles; under the same conditions as fig. 5. ×480.

Fig. 7.—A group of four scleroblasts lying undisturbed in the mesoglœa and each showing a secretion-knob. Stained with paracarmine and mounted in Canada balsam. ×480.

Fig. 8.—Two scleroblasts with associated collosclercs and vesicles, lying undisturbed in the mesoglœa with portions of three megascleres. Stained with paracarmine and mounted in Canada balsam. ×480.

Fig. 9.—A single scleroblast with associated vesicle and collosclere probably secreted by itself, lying undisturbed in the mesoglœa. Stained with paracarmine and mounted in Canada balsam. ×480.

Fig. 10.—Another scleroblast, isolated by teasing, with its associated vesicle and collosclere. Stained with paracarmine and mounted in Canada balsam. ×480.

Fig. 11.—A vesicle, with contained collosclere, partially torn away from a scleroblast by teasing. Stained with paracarmine and mounted in Canada balsam. ×480.

Figs. 12–16.—Five scleroblasts, showing stages in the growth of the secretion-knob. From teased preparations stained with paracarmine and mounted in Canada balsam. ×480.

Explanation of Lettering.—*col.*, collosclere; *meg.*, megasclere; *mes.*, mesoglœa; *nu.*, nucleus; *scl.*, scleroblast; *s.k.*, secretion-knob; *ves.*, vesicle containing collosclere.

On the Experimental Production of Congenital Goitre.

By Robert McCarrison, M.D., D Sc., F.R.C.P. (lately on Special Duty for the Study of Goitre and Cretinism in India).

(Communicated by Sir Victor Horsley, F.R.S. Received March 18, 1916.)

[Plate 12.]

The experiment herein described was undertaken at the Central Research Institute, Kasauli, India. It commenced on September 6, 1913, and terminated, owing to my recall to military duty for active service, on December 24, 1914. Having been on service for the past 18 months I have not hitherto had an opportunity to report it.

Object of the Experiment.

Its object was to determine the cause of congenital goitre and the conditions under which it developed in large animals, and to confirm and amplify the results I had obtained by previous experimentation on white rats.

It was consequently designed so as to subject the fœtuses of primiparæ to

highly toxic and goitrigenous agencies during intra-uterine life, and to contrast the results with those observed in the offspring of primiparæ which had as far as possible been protected from these influences.

Conditions of the Experiment.

For this purpose female goats of the first year, imported from a non-goitrous locality in the plains of India, were used; they were divided into the following classes for observation:—

Class A included 12 in which small goitres had been artificially produced. They were confined in a pen 10 yards square, and permitted to mix freely one with another. Throughout the experiment and during the whole period of gestation they were fed daily on cultures of bacteria from the fæces of goitrous individuals.

Class B included eight females, non-goitrous goats, which were isolated one from another by tethering them some distance apart. Four of them were also muzzled so as to exclude the possibility of infection by contaminated food, the muzzles being removed only at stated intervals when the animals were fed. The remaining four were not muzzled. No cultures were administered to any of them.

Class C included one young female goitrous goat, from which I had removed half the enlarged thyroid. The animal lived an entirely normal life throughout the period of the experiment.

The animals in all three classes were impregnated by the same non-goitrous male during the same season—*e.g.*, sometime after March 1, 1914, when the experiment proper commenced. Their conditions of life were in every other respect identical. Those in Classes B and C acted as controls to those in Class A.

The only factors, then, to which differences of results could be attributed were:—

(1) Intimate contact, in the case of animals of Class A.

(2) Muzzling, in the case of one half of those in Class B.

(3) The presence of goitre in the mothers of Classes A and C, and

(4) The administration of the cultures to those of Class A.

Results of the Experiment.

The results of this experiment are shown in the following Table:—

	No. of mothers.	No. of mothers goitrous.	No. of kids born.	No. of kids born alive and healthy.	No. of kids stillborn.	No. of kids stillborn, but fully developed.	No. of kids stillborn, macerated, hairless, and ill-developed.	No. of kids born with congenital goitre.
Class A—								
Receiving cultures ...	12	12	11	1	10	0	10	11
Class B (controls)—								
Muzzled	4	0	3	2	1	0	1	0
Unmuzzled	4	2	2	1	1	1	0	2
Class C—								
(Controls)	1	1	1	1	0	0	0	0

SUMMARY.

	Experimental.	Controls.
Healthy living kids	1, or 9 per cent.	4, or 66·6 per cent.
Stillborn, hairless, ill-developed kids	10, or 90 „	1, or 16·6 „
Stillborn, but fully developed kids	0, or 0 „	1, or 16·6 „
Congenitally goitred kids	11, or 100 „	2, or 33·3 „

It is clear from these results that there is a marked difference in the three classes of animals.

With regard to the mothers themselves: it was not observed that the goitres of those in the first and third classes underwent any considerable increase in size during pregnancy. Amongst the mothers in Class B, however, two of the unmuzzled animals developed small goitres during pregnancy.

With regard to their offspring: 100 per cent. in the first class were goitrous, while 90 per cent. were stillborn, macerated, hairless, ill-developed, and goitrous fœtuses, death having been intra-uterine (Plate 12, fig. 1).

In the second class, on the other hand, none of the offspring showed all these pathological characteristics at one and the same time. Three of the five kids born in this class were perfect in health and development, *e.g.*, 60 per cent. One had slight goitre which disappeared shortly after birth. A fourth was stillborn and goitrous, but as it was otherwise well developed, covered with a thick coat of healthy hair (fig. 2) and showed no signs of maceration, its death was probably extra-uterine. The fifth was born prematurely but was not goitrous. Its premature birth might well have

been due to influences quite outside the scope of the present experiment (fig. 3).

The kid born of the mother in the third class was perfectly healthy; its thyroid was palpable but there was no goitre.

Amongst the nine control animals, therefore, six had offspring; 66·6 per cent. of kids born were living and healthy, 33·3 per cent. were stillborn, while 33·3 per cent. were goitrous at birth. In neither of the two kids born with goitre did the enlargement reach the size of the majority in Class A.

Characteristics of the Fœtal Goitre.

(1) In all cases the congenital goitre of the offspring was markedly larger than that of the mother, sometimes as much as 20 times as large.

(2) Macroscopically they were of the appearance and consistency of normal kidney. On section the gland resembled a solid organ such as the liver, the cut surfaces freely exuding fluid which subsequent histological examination showed to be composed largely of thin blood-stained colloid.

(3) Microscopically the gland presented the appearances characteristic of very active secretion. The capillaries were congested, the alveoli small and lined with high columnar epithelium, which was often so high as to almost obliterate the acinar cavity. The cellular protoplasm was pale and vacuolated, the nuclei large and spherical, or small and irregular-shaped representing recent division forms. Evidences of nuclear division, of cell-multiplication and of new acinar formation were everywhere numerous. Thin, pale-staining colloid secretion filled the minute acini, permeated the interstices between the parenchyma cells and distended the lymph spaces, in some of which considerable accumulations of a more deeply-staining colloid were to be seen.

(4) Cultural methods of examination, both aërobic and anaërobic, showed these goitres to be invariably sterile.

Discussion and Conclusions.

Returning now to a consideration of the factors to which the differences of results in these animals are attributable, we have first to consider whether the free contact of those in the first class may have influenced the results. We have seen that goitre appeared in two of the eight control mothers of Class B and congenital goitre in the offspring of both of them, although the animals were prevented by their tethered position from intermingling one with another; such association is not a necessary factor, therefore, in the development of either acquired or congenital goitre. It may possess a favouring influence by increasing the infectivity of the site on which the

animals live. The infection is conveyed from one animal to another by some indirect means and not directly, as by contact.

The factor of muzzling appears to have a more important influence, since neither the muzzled goats nor their offspring developed goitre, whereas 50 per cent. of the unmuzzled goats did. The prevention of the entry into the alimentary tract of anything but clean food and water appeared to prevent the entry of the infecting agent. This being so, the infecting agent must have existed or generated in the plot of earth on which the animals were tethered and which their own alvine discharges had grossly contaminated—a conclusion which is supported by all reliable observations on man, animals, or fish. My previous researches and those of Gaylord on salmonoid fishes have shown that fish pollute the tanks, animals the soil, and man the dwellings where they are confined in such a way as to give rise to goitre. Confinement and fæcal pollution are the essential factors in its production; without them goitre does not arise. These conditions were fulfilled in the case of the muzzled and unmuzzled goats alike, but in the former infection could not occur, since muzzling closed its route.

The factor of the presence of goitre in the mother is of great importance in the genesis of congenital goitre in the offspring. Not only were 100 per cent. of the offspring of the goitrous mothers in Class A born with congenital goitre, but also congenital goitre occurred in two kids born of control mothers which had acquired goitre during their pregnancy.

The influence of cultures administered also appears to be great. The congenital goitres noted in Class A were, as a rule, of large size, while the offspring was almost invariably stillborn, macerated, ill-developed and hairless, an association of pathological characteristics which was not observed in the controls of Class B, or Class C, and attributable, I think, solely to the greater toxicity of the maternal blood in the animals of the former class. Whether this toxicity was due to the generation by the organisms contained in the cultures of toxic substances after their implantation into the intestinal tracts of the animals or to toxic substances contained in the original culture tubes does not materially affect the argument, since in either event these substances were the products of micro-organisms contained in the culture tubes and the true causal agents of the malady. The toxic products of these organisms, then, possess the property not only of inducing the development of large goitres in the fœtus but also of retarding fœtal development and bringing about its premature cessation.

The conclusion set forth in my former paper* is, therefore, confirmed.

Congenital goitre is due to the action on the fœtal thyroid of toxic

* 'Indian Journal of Medical Research,' vol. 2, No. 1 (1914).

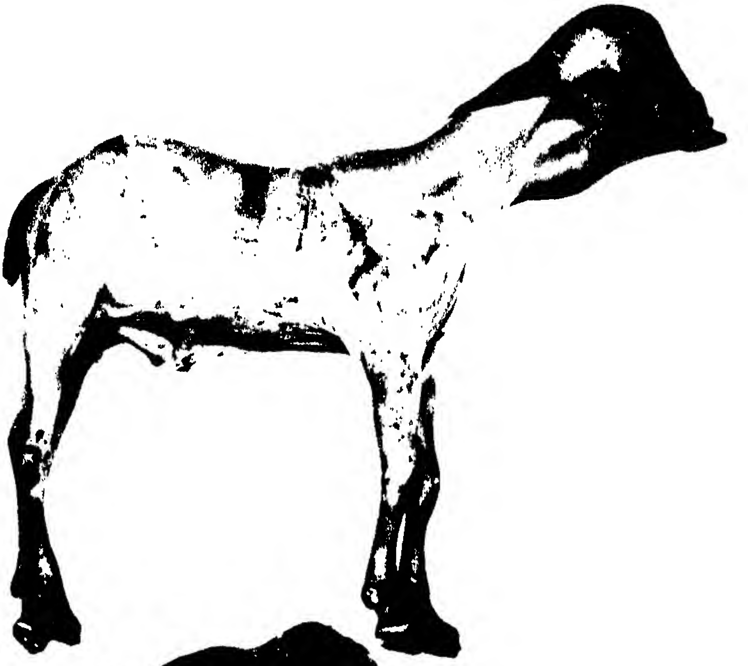


Fig. 1

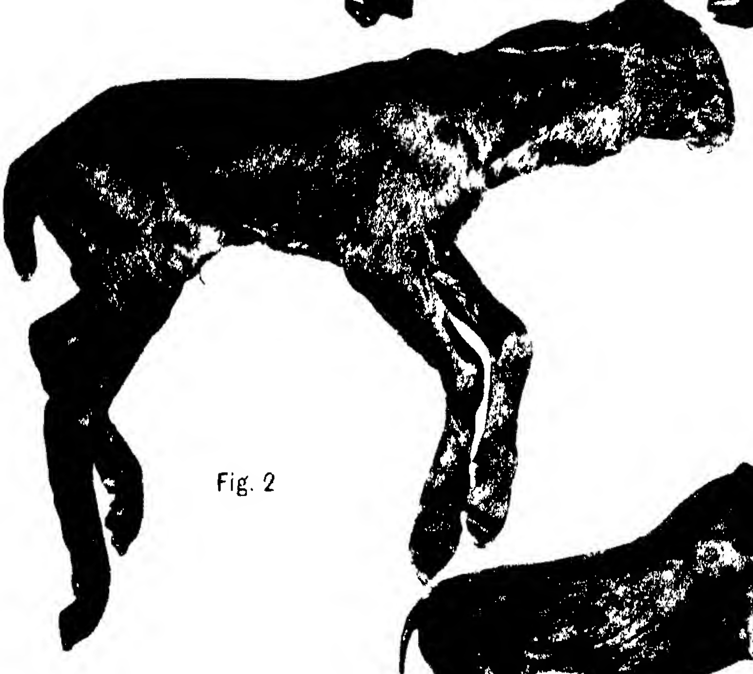


Fig. 2

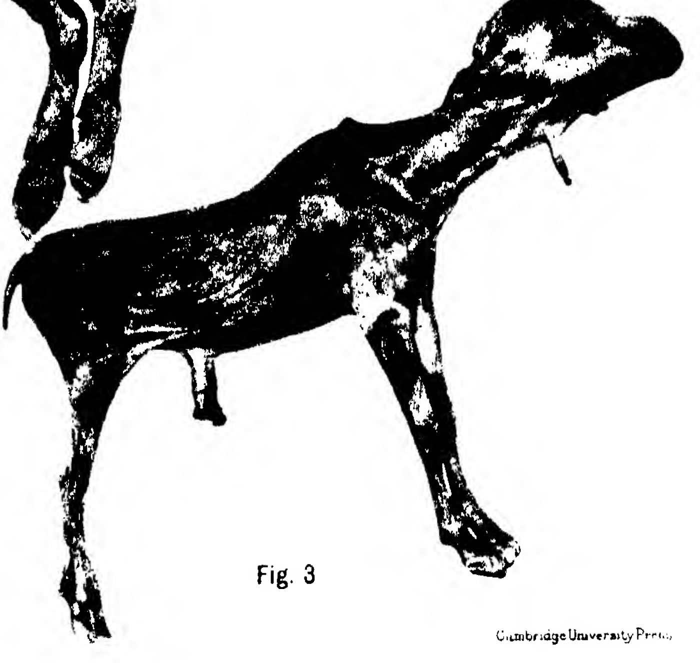


Fig. 3

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substances derived from the maternal intestine. These substances are the products of the micro-organisms originating in fæcally contaminated soil, which are conveyed to man and animals by infected food and water.

DESCRIPTION OF PLATE.

Fig. 1.—Stillborn severely-goitred kid, offspring of a goitrous mother (Class A) which consumed cultures from the fæces of goitrous individuals during pregnancy. Developmental defects are shown in the complete absence of hair and in the condition of the horny hoof. This case is typical of the 10 fœtuses of this class (A).

Fig. 2.—Stillborn goitred kid, offspring of an unmuzzled mother (Class B) which developed goitre during pregnancy. The kid is covered with a thick coat of healthy hair, and the hoofs are normal in appearance.

Fig. 3.—Fœtus of muzzled non-goitrous mother (Class B) showing no goitre.

The Ultra-Violet Absorption Spectra of Blood Sera.

By S. Judd Lewis, D.Sc. (Tübingen), B.Sc. (London), F.I.C.

(Communicated by Sir William Ramsay, K.C.B. Received March 18, 1916.)

At the end of the year 1913 there was introduced a new kind of sector spectrophotometer designed especially for investigation of the ultra-violet spectrum. The possibility of applying the new instrument to the furtherance of medical and physiological science was soon appreciated by the author, and in conference with Dr. C. E. Wheeler it was decided that a study of the ultra-violet absorption spectra of blood sera might lead to results which would be both valuable to science and applicable to clinical practice. The proposal was placed before the Beit Research Fund Committee, the trustees of a fund which had been placed at the disposal of the British Homœopathic Association by Mr. Otto Beit for purposes of scientific research. The necessary support was liberally given by the Association, and still further funds are allotted for continuing the work.

The absorption spectra of blood have engaged the attention of many able and distinguished workers, but the investigation has usually had reference to the visible spectra of hæmoglobin and other colourings and to derivatives of these. The work now to be described has for its object the investigation of the absorption spectra of blood sera in the ultra-violet region. The serum is freed as much as possible from corpuscles by the centrifuge, and the clear pale yellow liquid itself is studied with a view

to determining the various characteristics of the absorption bands and to finding how these may be accounted for.

The subject may conveniently be treated in three divisions:—

(1) Methods and Equipment.
(2) Absorption Spectra of Blood Sera.
(3) Review.

Methods and Equipment.

Most of the older methods of studying ultra-violet absorption spectra depend on taking a series of absorption spectra, obtained on passing light through layers of the substance varying in thickness or in concentration. The modern method is to employ a sector spectrophotometer in conjunction with a quartz spectrograph, and produce only one absorption spectrum resulting on the passage of light through a suitable layer of the substance, and to compare it with each member of a series of normal spectra differing from one another by known amounts in intensity only. Thus, a normal spectrum of one-third of the original intensity will match the absorption spectrum at those wave-lengths where one-third of the light is transmitted, that is, where two-thirds of the light are absorbed; and similarly for any other proportion.

The apparatus employed were: (*a*) A large size ("size C") quartz spectrograph made by Hilger, 1914 model. (*b*) A sector spectrophotometer. Two of these instruments have been employed in the course of the work. The one referred to in the opening of the paper is that introduced by Messrs. Adam Hilger, Limited. It is described by Lankshear in a paper in the 'Memoirs and Proceedings of the Manchester Literary and Philosophical Society' (vol. 58, No. 15, Part 3, pp. 1-12, 1914). The second instrument is a still later model made by Messrs. Bellingham and Stanley, Limited. It differs from the Hilger in its construction providing for the incident pencils of light being divided and brought together again by reflecting prisms instead of by refracting units, and in the "sector" being so constructed as to present an aperture which has the shape of a goemetrical sector, and so to give a constant exposure instead of an intermittent one. This avoids the necessity for correcting each batch of plates for the error due to intermittency. (*c*) A powerful spark lamp provided with nickel steel electrodes.

The experimental procedure adopted for the serum work is as follows:—

A photograph of the two juxtaposed spectra is taken with both sectors of the photometer fully open, and with nothing in either path save an

empty observation cell in each; or if a solution is to be examined, cells of the solvent are interposed. The two spectra should be identical, and thus show that the instruments are in proper adjustment. This photograph is conveniently called a "test band."

One of the empty cells is then replaced by a similar cell containing a layer of the serum, the thickness of which has been determined as accurately as possible, say to the one-thousandth part of a millimetre. This is a matter of great importance, as the layer is usually less than one-fifth of a millimetre thick. Thus a possible error of one-half per cent. is assumed, and this postulates a similar error in measuring the magnitude of the absorption.

The photographic plate is lowered in the camera to expose a fresh strip of the plate. The sector in the other path of light is set at the aperture 0·9, by which the intensity of the light is reduced to 0·9 of the original intensity, and a fresh photograph is taken. The plate is again lowered, the sector set at the aperture 0·8, and another photograph produced. The process is repeated with as many more sectors as desired, usually not less than eighteen and not more than fifty-four. The plates adapted to the spectrograph camera measure 10 inches by 4 inches and accommodate 18 photographs. Finally another test band is registered to show that the adjustment of the instrument remains unaltered.

The plate having been developed and dried, the points of equal intensity in each pair of spectra are marked by small dots. The wave-length of each of these points is determined by reference to a corrected wave-length scale photographed on the plate, and tabulated along with the corresponding sector aperture, that is, along with the corresponding intensity of the light transmitted. In accordance with the usual practice this is applied to determine the absorptive power of the substance in terms of the quantity $\log (I/I')$, known as the "extinction coefficient," where $I =$ the initial intensity, and $I' =$ the intensity of the unabsorbed light.

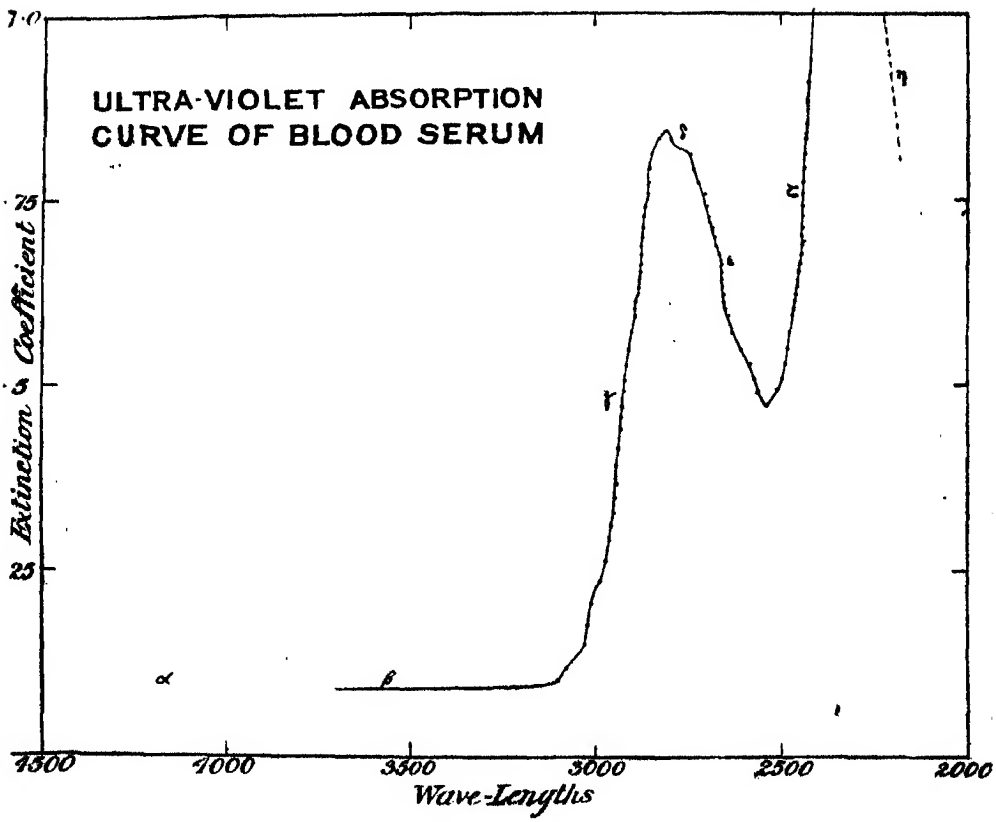
In view of serum being a solution, the composition of which is variable and comparatively unknown, the extinction coefficient must apply to the serum in some form arbitrarily chosen as a standard. The standard adopted is a layer of serum one-tenth of a millimetre thick. When the substance is employed in a thickness or concentration other than that prescribed for the standard, the extinction coefficient must be calculated according to the formula $cd/c'd' . \log (I/I')$, where d is the standard thickness, c the standard concentration, d' the thickness employed, c' the concentration employed.

Since existing work on the spectra of blood is recorded in wave-lengths, it is desirable to continue this in order to correlate the new work with that

of the past. The "absorption curve" is plotted therefore with extinction coefficients as ordinates and wave-lengths as abscissæ.

Absorption Spectra of Blood Sera.

As no investigation of the ultra-violet absorption spectrum of blood serum appears to have been made hitherto, it is desirable, first of all, to study the absorption curve of normal serum in detail.



The varying intensity of the absorption is expressed in the curve shown in the figure. For purposes of reference it is convenient to describe the several well characterised parts of the curve as "sections," and to designate them by the letters of the Greek alphabet. The first section, α, coincides with the visible region of the spectrum. The next section, β, covers the region of least extinction, extending from the borders of the visible to a wave-length of about 3100, where it meets the foot of the steep ascent from this point to the head of the band at about 2800, section γ. The descent

on the other side of the band comprises two sections; section δ describes a short, nearly vertical fall from the head at wave-length about 2800, and an abrupt projection towards the ultra-violet to a point where the curve turns sharply downwards again. Section ϵ extends from this point to the depression at wave-length about 2540, whence section ζ rises steeply to a high value at a wave-length about 2400. A further section, η, has been observed with three or four pathological specimens, and is indicated in the figure by a dotted line.

The peculiarities of the central band call for special attention. Section γ exhibits slight irregularities of form which are found to be constant. Among these, the most evident are slight step-like prominences at about a quarter and half-way down from the top, and one very near the bottom, which is often well developed. Section δ includes with the upper part of section γ a small area of elongated shape, which varies somewhat in altitude. Experience shows that the section ϵ is the part most subject to variation (*vide infra*). Section γ seldom varies much except when, as in some pathological sera, the greater changes in δ or ϵ extend their influence across the band. Section β is subject to some variation; section ζ has rarely been found to alter materially.

Between sixty and seventy specimens of normal blood have been examined, with results which are practically constant. The general character of the curve has never altered. In the course of their study, the various peculiarities of the absorption spectrum have become recognised gradually, and the method of procedure improved from time to time so as to develop the several parts of the curve. The chief conclusions arrived at are recorded in the description already given.

Sex and age have not so far found any well defined expression in the properties of the absorption curve. On the other hand, it cannot be said that there is no general differentiation. The central band appears to be slightly narrower in the female, and to be shallower in the child, but one merges into another so gradually that nothing more precise can be said until a considerable number of specimens have been very carefully compared under the best conditions, taking full advantage of all earlier experience.

Inasmuch as serum is a solution of a mixture of substances in somewhat variable proportions, smooth absorption curves comparable with those obtainable with pure substances cannot be expected. The serum curve is rather the resultant of several superposed curves, each characteristic of some constituent of the fluid. It becomes of interest, therefore, to separate these members and to determine their individual absorptions with

a view to building up the resultant curve from its parts. This problem is one of great importance, for only in such manner can one hope to determine what constituents of the serum are affected in any given disease. It has already been solved in general terms and its precise study is in progress. It has been found that the chief central band is due almost entirely to proteins. The constancy of the form of section ζ points also to the same constituents. This has been ascertained in the following manner:—A portion of serum is weighed and mixed with alcohol, whereby the proteins are precipitated, leaving the non-proteins in solution. The operations are conducted so that after dilution 100 volumes of solution contain the non-proteins from 1 volume of serum. A portion of the solution is examined in a 2 cm. cell; this layer corresponds with the non-proteins in a film of serum 0·2 mm. thick, so that the absorption curve requires but little correction to make it comparable with that of the film of serum usually employed. The effect of the solvent is eliminated in the manner already indicated. The distribution and proportion of that part of the absorption by serum due to non-proteins may then be readily appreciated. It is found to consist essentially of a general absorption which is slight from the visible to a wave-length of about 2100, and then to increase rapidly.

The proteins are washed with 90-per-cent. alcohol, separated in the centrifuge and dissolved in such a quantity of water that 100 volumes of the solution contain the proteins from one volume of serum. The manner of examination is similar to that for the solution of the non-proteins. The form of the resulting curve approximates that for the original serum, showing that the proteins account for most of the absorption observed with serum.

Horse serum (three specimens) has also been the subject of inquiry. Its absorption curve is very similar to that of human serum, but (*a*) the depression is at wave-length 2510 instead of 2540, (*b*) the amplitude from this point to the head of the curve at 2800 is rather greater, and (*c*) the first step-like prominence in section γ is somewhat lower and decidedly more pronounced, so that the curve for the protein band is in general of rather larger dimensions than is the case with human serum.

Egg white (one specimen) was dealt with in similar manner for comparison. The curve of the protein band is more symmetrical in form. The sections ϵ and ζ meet at 2540 as with human serum.

Pathological.—A considerable number of pathological specimens have been examined, with some significant results. As would be expected, abnormality is confined to certain diseases; and again, as must be anticipated, the magnitude of the disturbance is usually small. One cannot look for severe

distortion of the curve, for such would imply great modification of the proteins or other constituents of the serum and point to very serious changes in the condition of the patient. Although about 120 specimens of pathological sera have been studied, it would be premature at this stage to do more than make a few general remarks.

The first observation is a general one and very significant, namely that it is the section ϵ which is most usually disturbed. It is, moreover, the part which shows most variation in normal or so-called normal serum, and it is also here that the most marked differences between human serum and horse serum, and between either of these and egg albumin, are to be found. It appears, therefore, that modification of this part of the absorption band corresponds with some sensitive constituent which varies either in proportion or in constitution in consequence of comparatively slight changes in the condition of the subject.

Some thirty specimens of blood have been examined in connection with typhoid, and the results are very encouraging. The chief effect observed is that the point of least absorption value between the sections ϵ and ζ is shifted from 2540 to 2510, and at the same time raised slightly.

This result has been arrived at in two ways: First, a series of three specimens of blood was taken from each of six soldiers; (*a*) normal, immediately before inoculation against typhoid; (*b*) 41 hours after the first inoculation; (*c*) 20 hours after the second inoculation, 11 days later. The serum was separated and examined in the usual way, and the above-named effect was observed in five out of six cases. Blood from 11 cases of actual or suspected typhoid was examined. In six instances the above effect obtained fully; in two, the displacement was to 2530 only; in two, the position was unchanged. In one, described as clinically a typical case of typhoid and as having failed three times to give the Widal reaction, the movement was in the opposite direction, to 2550.

In most cases there is a reduction in the amplitude of the curve between the depression at 2540 and the head at 2800.

Another modification observable in the inoculation cases is that the step-like prominence at the bottom of section γ is somewhat greater after inoculation than before in five cases out of six. The sixth case is also the exception with regard to the displacement modification mentioned above.

Scarlet fever has proved very interesting. It exhibits a more or less strong disturbance in the protein band in about half of the 33 cases which have been examined. The change is not constant either in quality or quantity, but it is always in the central protein band; usually section ϵ is

most strongly modified, but the whole band may be greatly reduced, with its head thrown towards the extreme ultra-violet.

Tuberculosis has been the subject of inquiry in 27 cases; 10 of them exhibit a band of slightly increased extinction in section β between wave-lengths 3200 and 3500. Another ten show a tendency to some ill-defined absorption in the same regions, or an increase in the step-like prominence at the foot of section γ. The remaining seven were all "mild" cases, and caused no special absorption. However, some others similarly described were not distinguishable from those marked "severe."

In four cases of anæmia the serum presented no abnormality. Miscellaneous cases of rheumatism, rheumatoid arthritis, cirrhosis of the liver, etc., have afforded irregularities in the curve, but confirmation is needed to establish their significance.

Review.

So few natural substances of unknown constitution appear to have been submitted to direct examination by ultra-violet absorption spectrography, that the success attending the present investigation of serum is the more gratifying.

Practically all the properties of the absorption curve of normal serum have proved to be constant and characteristic, while there is enough variation in minutiæ to stimulate a closer investigation, with a view to ascertaining the range and causes of the variations, and the much greater, though still small, changes associated with certain pathological conditions make the inquiry all the more urgent and interesting. The method lends itself to the purposes of clinical practice, for so small a quantity as four or five drops of blood collected in a capillary tube suffice for a complete examination in the ordinary way. Again, no preparation whatever of the specimen is necessary except to separate the serum in the containing tube by means of the centrifuge, and then to transfer it to the observation cell.

It is improbable that any important improvement will be made in the qualitative properties of the absorption curve as described above. The extinction coefficients are, however, only approximately quantitative. The greatest hindrance to reaching the final goal has been the lack of a sufficiently perfect spectrophotometer. It is worthy of emphasis that the work described has been done on the two most modern and most accurate instruments available, which have proved adequate for most academic requirements. But the exacting demands of the serum work reveal the necessity for one still finer. The author has designed a new photometer, which is now under construction, and will, it is hoped, supply the need.

Precision, capacity for truly quantitative measurements, uniformity of adjustment and rapid working, are among the most urgent desiderata. With the new instrument former work will be confirmed and rendered quantitative, while it is hoped to deepen and extend it in such manner as to lay sure the foundations of this new branch of practical science.

An Experimental Investigation into the Rôle of the Blood Fluids in the Intracellular Digestion of Certain Bacteria and Red Blood Corpuscles.

By S. R. Douglas, M.R.C.S., L.R.C.P. (Lond.), Captain I.M.S. (Retired), 1st Assistant, Bacteriological Department, Medical Research Committee, National Insurance Act.

(Communicated by Sir Almroth Wright, F.R.S. Received June 3, 1916.)

(From the Inoculation Department, St. Mary's Hospital, Paddington, W.)

Any observer who may have carried out a number of experiments on phagocytosis *in vitro*, especially when some members of the *coli* group, or the gonococcus, have been the microbes under observation, cannot fail to have been struck by the marked intracellular digestion which is seen in the microscopical preparations made in the course of these experiments.

This intracellular digestion is evidenced by many of the organisms ingested by the leucocytes appearing as ill-stained, swollen shadows lying in vacuoles.

Further, several independent workers in this laboratory, when working out the opsonic index in the case of glanders, noticed that those glanders bacilli which had been ingested by the leucocytes after being acted on by the normal human serum showed from their appearance in the opsonic films many more signs of digestion than those which had been acted on by the patient's serum, and this was the case quite independently of the value of the opsonic index of the patient's serum, as is shown by the fact that on occasions when this was especially noted the opsonic indices were 2·6, 2·7, 1·7, 2·3, and 0·96 respectively. Another observation bearing on this subject, and one which many workers may have noticed, is that the gonococci seen in the leucocytes contained in specimens of gonorrhœal pus are sharp cut, taking the stain evenly and deeply, whereas in specimens made for the determination of

opsonic indices to this microbe, however perfect the bacterial emulsion used may be, many of the organisms seen inside the leucocytes are so much digested as to make the counting of the individual cocci, necessary to obtain the average per leucocyte, a more than usually irksome task.

In view of the fact that there is present in every serum a very marked antidigestive property, namely, the antitryptic power, it would appear almost paradoxical that the serum might play a favourable *rôle* in a digestive process, yet on consideration it is quite conceivable that in the case of bacteria or red blood cells the serum might, by some action short of bacteriolysis or hæmolysis, render such bodies more permeable by the digestive fluids, and in this way favour their digestion.

Rosenow was the first to draw attention to this question, for in working with the pneumococcus he found that the amount of digestion which took place after these organisms had been ingested by the leucocytes varied considerably in different bloods, and he came to the conclusion that this variation was due to a property of the serum which acted on the leucocytes—not on the organisms—stimulating them to increased digestive efforts.

The experiments here detailed have forced upon me the conclusion that there is a property of the blood fluids independent of the opsonic power, which acts directly on the micro-organisms, or, as the case may be, on the red blood cells, rendering them more easily digested by the leucocytic ferments.

Experiments Made to Ascertain whether Red-Blood Cells or Bacteria which had been Acted on by Serum were capable of being Digested by Solutions of Trypsin or Leucoprotease.

Preliminary experiments having shown, firstly, that the author's serum had no hæmolytic power in regard to washed ox red blood corpuscles; secondly, that when washed ox red blood corpuscles were mixed either with his defibrinated blood, or with a mixture of his washed corpuscles and serum, large numbers of the ox red blood corpuscles were ingested by the leucocytes, and in them rapidly digested; thirdly, that even strong trypsin solutions showed no digestive action on washed ox red blood corpuscles, the following experiment was made:—

After washing ox red blood corpuscles free from all serum by repeated centrifuging with normal saline solution, a 10-per-cent. suspension was made. A series of tubes were now filled in with the following mixtures:—

Tube I.—One volume of 10-per-cent. suspension of ox red blood corpuscles and 2 volumes of fresh serum (S. R. D.'s).

Tube II.—One volume of 10-per-cent. suspension of ox red blood cor-

puscles and 2 volumes of serum (S. R. D.'s), which had been heated to 60° C. for 18 minutes.

Tube III.—One volume of 10-per-cent. suspension of ox red blood corpuscles and 2 volumes of normal salt solution.

These tubes were then incubated at 37° C. for one hour, after which, by repeated centrifuging and washing in normal saline solution, all traces of serum were removed. The red blood corpuscles thus obtained were then suspended in a volume of salt solution equal to the original volume of the suspension of ox red blood corpuscles, and an equal volume of a 1 in 10 trypsin solution was added. The tubes were again placed in the incubator at 37° C. for three hours, and after that period were put in the ice chest, remaining there overnight.

On examination the next morning, Tube I, that is the tube in which the ox red blood corpuscles had been acted on by the unheated serum, showed marked digestive changes, evidenced by hæmolysis and change of colour of the liberated hæmoglobin, whereas in the case of Tubes II and III, in which the ox red blood corpuscles had been brought in contact with heated serum or with normal saline solution before the addition of the trypsin, no digestion had taken place.

The conclusion that is drawn from this experiment, which was repeated on several occasions with identical results, is that the unheated serum acts in some way on the red blood corpuscles, so as to render them susceptible to digestion by trypsin.

Further experiments, with exactly similar results, were made, in which a leucoprotease solution was substituted for the trypsin solution. It was also found that normal rabbit's serum, although without any hæmolytic action on human red blood corpuscles, had the power of, in some way, acting on these cells, rendering them susceptible to digestion by solutions of trypsin.

However, human red blood corpuscles, which had been brought in contact with rabbit's serum which had been heated to 60° C. for a few minutes, were quite unaffected by such trypsin solutions.

Substituting bacteria for red blood corpuscles, a series of experiments showed that, in the case of a strain of *B. Friedländer* and some other coliform organisms, a very distinct digestion, evidenced by the loss of opacity of the emulsion, was brought about when such organisms had been previously acted on by unheated serum and afterwards treated with solutions of trypsin or leucoprotease, whereas when the organisms had been treated with serum heated to 60° C., or simply suspended in normal salt solution, both trypsin and leucoprotease solutions were quite inert.

These experiments show conclusively that the blood fluids modify the

red blood cells, or, as the case may be, the bacteria, in such a manner that they undergo digestion when brought into contact with solutions of trypsin or leucoprotease, solutions which have been found to be quite inert on suspensions of red blood cells or bacteria which had been previously acted on by heated serum or simply suspended in normal saline solution.

It is proposed to call this property of the serum the "protryptic" property of the serum, indicating that the serum by this action prepares the organisms or red blood cells for digestion by the leucocytic digestive fluids.

Experiments made to Ascertain whether only those Bacteria which had been Phagocytosed in the Presence of Unheated Serum were Liable to Undergo Intra-leucocytic Digestion.

Having found that plague bacilli are taken up by the leucocytes in considerable numbers, even in the presence of heated serum, an emulsion in normal saline solution of *B. pestis* was made from a 24-hour-old agar culture.

Human blood corpuscles (S. R. D.'s) were washed free from serum by centrifuging them in several changes of salt solution, and two samples of serum (S. R. D.'s) were obtained, one of which was heated to 60° C. for 10 minutes.

Two capillary tubes, such as are used in ordinary opsonic estimations, were now filled in with the following mixtures:—

Tube I contained:—

2 volumes of the washed corpuscles.
2 volumes of the unheated serum.
1 volume of the emulsion of *B. pestis.*

Tube II contained:—

2 volumes of the washed corpuscles.
2 volumes of the serum heated to 60° C. for 10 minutes.
1 volume of the emulsion of *B. pestis.*

These tubes were then incubated at 37° C. for four hours, after which films were made of samples of their contents, fixed in a saturated solution of corrosive sublimate and stained with carbol-thionin.

On examination, the microscopical preparations made from both tubes showed abundant phagocytosed bacilli, but whereas in the case of those made from Tube II, in which heated serum had been used, the bacilli lying in the leucocytes were deeply stained and perfectly sharp cut in appearance, in those made from Tube I, in which unheated serum had been used, the bacilli taken up by the leucocytes were almost completely digested, appearing as swollen, ill-staining shadows, frequently lying in well marked vacuoles.

This experiment was repeated again and again, sometimes with slight modifications, such as allowing the serum to act on the bacteria for varying periods before the washed corpuscles were added, but the result was always the same, namely, intra-leucocytic digestion could only be demonstrated when the organisms had been acted on by fresh unheated serum.

These experiments, although they furnished data showing conclusively that intra-leucocytic digestion only took place in the presence of unheated serum, were unsatisfactory, in that they failed to give any idea as to the proportion of organisms which were digested after being taken up by the leucocytes. The following experiments were therefore undertaken with the view of elucidating this point.

Phagocytic mixtures consisting of, on the one hand, washed corpuscles, unheated serum, and an emulsion of plague bacilli, and, on the other hand, of washed corpuscles, heated serum, and an emulsion of plague bacilli, were incubated at 37° C. in a water-bath.

At varying intervals samples of these mixtures were withdrawn and microscopical preparations were made and the number of bacilli contained in 100 leucocytes was ascertained.

In the case of the phagocytic mixture which contained the unheated serum it was found that the number of bacilli that could be recognised in the leucocytes became smaller and smaller the longer the tubes were incubated, at any rate up to a period of four hours, and from these figures it was possible to make a rough estimation of the number of organisms that had been digested.

In the case of the mixture which contained the heated serum the result was completely different, since each successive sample showed that the leucocytes contained larger and larger numbers of bacteria and these appeared normal as regards both their shape and staining reaction.

The details of one such experiment are here given.

A very thick emulsion of plague bacilli, from a 24-hour-old agar culture, was made in normal saline to which 1 per cent. of formalin had been added. The emulsion was kept at room temperature for about one hour to allow the formalin to act on the bacilli.

This procedure, which killed the organisms, was necessary, as when similar experiments were made with emulsions of living plague bacilli it was found that after incubation at 37° C. for some hours a proportion of the bacilli which had been ingested by the leucocytes were capable of multiplying, and in consequence of this the results obtained were irregular and fallacious.

This thick emulsion was now diluted 100-fold with normal saline, so that the concentration of formalin in the final phagocytic mixture given below was

reduced to 1 in 6000, a strength which has little or no inhibitory effect on phagocytosis.

Two small test-tubes were now filled in with the following mixtures:—

Tube I. 250 c.mm. of washed corpuscles.
250 c.mm. of unheated serum.
100 c.mm. of the emulsion of plague bacilli.

Tube II. 250 c.mm. of washed corpuscles.
250 c.mm. of serum heated to 60° C. for 20 minutes.
100 c.mm. of the emulsion of plague bacilli.

Both tubes were incubated in a water bath at 37° C. and after stated intervals samples from each were removed with a capillary pipette. From these, films were made which were fixed with a saturated solution of mercuric chloride and stained with carbol thionin.

The number of bacilli contained in 100 leucocytes was now estimated.

This was by no means an easy task in the case of some of the films made from the samples taken from tube I, in which owing to the presence of the unheated serum the bacilli were undergoing intraleucocytic digestion.

The numbers given below include not only the organisms which, because of their perfect shape and staining, were deemed unaffected by the leucocytic digestive fluids, but also many which showed signs of commencing digestion.

Time of incubation.	Number of bacilli in 100 leucocytes in samples from Tube I, containing unheated serum.	Number of bacilli in 100 leucocytes in samples from Tube II, containing heated serum.
15 minutes	330	80
30 "	280	140
1 hour	210	210
2 hours	95	280
3 "	57	250
4 "	27	300

On examining the figures thus obtained, assuming that no more bacilli were ingested by the leucocytes after the first 15 minutes' incubation, the only conclusion that can be drawn is that 90 per cent. of those organisms which were ingested in the first 15 minutes have been so completely digested during the succeeding 3¾ hours that they no longer can be recognised as bacteria.

But most probably what does happen is that the leucocytes continue to take up microbes to some extent during the whole period of incubation, so that in reality even a greater percentage than 90 per cent. of those microbes which were ingested by the leucocytes in the first 15 minutes have been

completely digested during the succeeding $3\frac{3}{4}$ hours the phagocytic mixtures were incubated.

When the figures given by the films made from the samples taken from tube 2, which contained heated serum, are examined, it is found that, instead of the number of bacilli per 100 leucocytes diminishing, there is a steady increase until, after the incubation of this tube had continued for four hours, the number of organisms found to be ingested in 100 leucocytes approximated to the number ingested through the action of the unheated serum in 15 minutes.

Further, even after incubation had continued for four hours the bacilli lying in the leucocytes showed practically no signs of digestion.

Summary and Conclusions.

These experiments showed that, as regards the particular bacteria and red blood corpuscles, and also as regards the blood fluids used in carrying them out :—

1. The blood fluids have the property of influencing the digestion of such bodies as red blood corpuscles and bacteria taken up by the leucocytes.

2. This action of the blood fluids is quite independent of the opsonic action, this being shown by the fact that intracellular digestion may be more marked as the result of the action of a serum of lower opsonic power than of a serum of much higher opsonic power.

In these two conclusions the author is in complete agreement with the conclusions drawn by Rosenow.

3. The power of the blood fluids to prepare such bodies as red blood cells or bacteria for digestion by solutions such as trypsin and leucoprotease, or by the digestive fluids which are secreted after such bodies are ingested by the leucocytes, is not, as stated by Rosenow, due to stimulation of, or an action on the leucocytes, but is due to a direct action on the bacteria, or, as the case may be, the red blood corpuscles. This is demonstrated by those experiments in which the red blood corpuscles or bacteria, after being brought in contact with fresh serum, which was subsequently removed, were found to be digested by solutions of trypsin or leucoprotease, solutions which had been previously shown to be quite without action.

4. Heating the serum to 60° C. destroys the property of the serum to prepare such bodies for digestion, at any rate in the case of normal serum.

5. It is proposed to name this property of the blood fluids the "protryptic" power of the serum, seeing that it prepares such bodies as red blood corpuscles and bacteria for solution by the digestive fluids secreted by the leucocytes or by solutions of trypsin.

Some Photochemical Experiments with Pure Chlorophyll and their Bearing on Theories of Carbon Assimilation.

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CONTENTS.

Introduction.

The main features of the processes of carbon assimilation are that the green plant under the influence of radiant energy takes in carbon dioxide, evolves oxygen, and in the majority of cases produces carbohydrates. The only aspect of these processes which has been subjected to detailed investigation is the intake of carbon dioxide, our knowledge of which is due to the extensive researches of F. F. Blackman and of R. Willstätter. Blackman has investigated the factors, such as light, temperature, carbon dioxide supply, which influence the intake of carbon dioxide by the ordinary green leaf, while Willstätter has extended Blackman's work by including quantitative investigation of the amount of chlorophyll.

When we turn, on the other hand, to the question of the actual physical and chemical processes of carbon assimilation we find many hypotheses, but few facts. We are acquainted with the primary facts of the intake of carbon dioxide and the formation of carbohydrates, etc., but we have no definite knowledge of the intermediate processes concerned.

Since the hypothesis of Baeyer in 1870 it has been generally supposed that carbon dioxide and water are synthesised to carbohydrates under the influence of light and in presence of chlorophyll, and that formaldehyde is an

intermediate product in this synthesis. Attention has consequently been directed to the detection of formaldehyde either in leaves themselves or in chlorophyll-containing systems outside the plant.

It is not possible in this paper to deal with all the work that has been published on this question. Below we give in tabular form a summary of these investigations which will serve to make clear the incompleteness of the researches and the lack of concordance in the results obtained.

In regard to experiments conducted outside the plant an important fact, with which we are now acquainted, was overlooked, namely, that the crude chlorophyll used in such experiments was really a very impure substance. Our knowledge of the chemistry of chlorophyll is now well advanced as a result of Willstätter's researches. We know that the chloroplasts contain two green and two yellow pigments. These four pigments together with a much larger quantity of colourless substances are extracted by the solvents used by the earlier investigators, and are present in the crude chlorophyll obtained by evaporating down such an extract. A good criterion of pure chlorophyll is that it is insoluble in petrol-ether, and even in a moderately impure condition is precipitated by it. It is therefore significant that most investigators, including even the latest workers, Wager (1914) and Ewart (1915), have used chlorophyll soluble in petrol-ether. In view of this it is possible that the various results obtained by those workers may be due to some extent to the impurities present in the crude chlorophyll and not to the chlorophyll itself.

In order to distinguish in the reactions observed by previous workers between those due to chlorophyll itself and those due to impurities either in the pigment or in the reagents, we have employed pure chlorophyll. We have also borne in mind the possibility of the photochemical production of formaldehyde by the solvents used.

So long as our knowledge of the chloroplast is as incomplete as it is at present, particularly as regards the distribution and components of the heterogeneous system which it constitutes, it is impossible, from experiments conducted outside the plant, to draw conclusions in regard to photochemical reactions taking place in the leaf.

Willstätter has shown that the molecule of both "chlorophyll *a*" and "chlorophyll *b*" consists of a chromogen complex combined with two ester groups so that the formula of "chlorophyll *a*" can be written

$$(MgN_4C_{32}H_{30}O)(COOCH_3)(COOC_{20}H_{39})$$

and that of "chlorophyll *b*"

$$(MgN_4C_{32}H_{28}O_2)(COOCH_3)(COOC_{20}H_{39}).$$

Table I.

Author.	Supposed changes produced under illumination.			Control experiments in dark.
	In a chlorophyll, water, CO_2 system.	In a chlorophyll, water, O_2 system.	In a chlorophyll, water, N_2 system.	
Pollacci, 1902	HCHO, H_2 and CH_4 formed. CO_2 absorption not examined	Not examined	Not examined.	
Usher and Priestley, 1906 and 1911	HCHO and H_2O_2 formed. Chlorophyll bleached. CO_2 absorption not examined	Not examined	Not examined.	
Schryver, 1910	HCHO formed. CO_2 absorption not examined	Not examined	Not examined.	
Warner, 1914	No trace of HCHO formed. No bleaching. CO_2 absorption not examined	HCHO and an oxidising substance formed. Action not accelerated by traces of CO_2 but by H_2O. Chlorophyll bleached	No HCHO. No bleaching	CO_2 and O_2 not examined. No HCHO.
Wager, 1914	Products not examined. Bleaching not examined. No CO_2 absorption detected	An aldehyde and a volatile oxidising substance formed. Chlorophyll bleached. Considerable O_2 absorption	No change	O_2 no absorption, no bleaching. CO_2 not examined.
Ewart, 1915	No HCHO and no reducing substance. Considerable CO_2 absorption	Large amounts of CH_2O formed. Chlorophyll bleached. Bleaching accelerated by traces of CO_2. O_2 absorption not examined	A dry film showed no change	CO_2 gave same as in light. O_2 and N_2 not examined.
Chodat, 1915............	HCHO and H_2O_2 formed. No mention of bleaching. CO_2 absorption not examined	Not examined	Not examined	Not examined.

As regards photochemical reactions we have mainly dealt with those which affect the chromogen complex of the chlorophyll molecule. In regard to these we have found no qualitative difference in relation to the source of light used, whether daylight, an arc lamp, or ultra-violet light from a quartz mercury-vapour lamp. For the sake of convenience and rapidity of work we have employed a mercury-vapour lamp since it gives a very intense and constant illumination.

The present investigation does not in any way claim to deal adequately with the photochemistry of chlorophyll. Its object has been to deal with the question as to how far certain observations described by previous investigators can be justly used in support of their theories. The production of formaldehyde by crude chlorophyll is used, for instance, by Usher and Priestley as the basis of their theory of carbon assimilation; also the supposed formation of xanthophyll from chlorophyll *in vitro* is used by Ewart in support of his contention that chlorophyll combines with carbon dioxide with the production of xanthophyll and oxygen, the xanthophyll being oxidised in presence of water to produce hexose sugars.

Chlorophyll Extraction and Purification.

The method used for the extraction and purification of chlorophyll is described in detail by Willstätter and Stoll (13, pp. 133–135; see also Jörgensen and Stiles (3)). The principle of this method for the isolation of chlorophyll is that suggested by G. G. Stokes in 1864, by which substances are separated from one another by their distribution between two immiscible solvents.

The material used consisted of nettle leaves dried at 30 to 40° C., and ground to a fine powder.

The chlorophyll and accompanying substances are extracted with 80-per-cent. acetone, and from this solution they are transferred to petrol-ether of S.G. 0·64 to 0·66. From this petrol-ether solution the colourless impurities are removed by washing with 80-per-cent. acetone. The xanthophyll is next removed by washing the petrol-ether with 80-per-cent. methyl alcohol.

Pure chlorophyll is insoluble in petrol-ether, but is soluble if small quantities of other substances, as, for instance, methyl alcohol, are present. The last traces of methyl alcohol and acetone have therefore to be removed by repeated washing with water. The chlorophyll is then precipitated generally as a fine suspension while the carotin remains in solution. The chlorophyll is obtained by filtering off the suspension through a layer of powdered talc, and is purified by dissolving it in pure, redistilled ether. It is

reprecipitated with petrol-ether, until finally a blue-black microcrystalline powder is obtained, which is a mixture of the two chlorophylls *a* and *b*.

As the purification of chlorophyll is somewhat difficult it is always advisable to test the purity of a chlorophyll preparation before use. We therefore give below the criteria of purity of the pigment, more particularly because some investigators, Ewart (2), for instance, state that Willstätter's methods have been followed, although it is quite clear that the product which they describe as chlorophyll cannot have been of even moderate purity.

Criteria of Purity of Chlorophyll.

1. *The substance should be free from yellow pigments and colourless impurities.*—Fortunately, in Willstätter's method of purification, the yellow pigments and colourless impurities are eliminated together, so that, generally, chlorophyll free from the yellow pigments is also free from the colourless impurities which are present along with the pigments in the chloroplasts. It is sufficient, therefore, to test for the presence of the yellow pigments.

This test is based on the fact that, by strong alkalis, chlorophyll is saponified to the water-soluble salts of the acids called chlorophyllins, while the yellow pigments are not attacked by alkalis. A 30-per-cent. solution of potassium hydroxide in methyl alcohol is therefore added to an ethereal solution of chlorophyll, and water added after saponification is complete, The green chlorophyllin salts are then found in the aqueous alkaline layer and any yellow pigments present will immediately be detected by the colour they give to the ethereal layer.

Hence, if the chlorophyll is pure, the ethereal layer should remain colourless after such a saponification test.

2. *The chlorophyll itself should remain unaltered during its extraction and purification.*—The changes in the composition of chlorophyll most likely to occur during its preparation are—

(*a*) The replacement of the magnesium by hydrogen, giving the magnesium-free derivative, phæophytin. This takes place in an acid medium. The presence of phæophytin is easily discovered, as it gives in solution a characteristic absorption spectrum containing two lines in the green, one just before the Fraunhofer line E, and one between the lines E and F. These lines are not found in the chlorophyll spectrum, nor in the absorption spectrum of a freshly prepared leaf extract which contains no phæophytin.

Pure chlorophyll should therefore give in solution an absorption spectrum similar to that from a freshly prepared leaf extract.

(*b*) Allomerisation. Particularly in water-free media, chlorophyll frequently undergoes a change, called by Willstätter allomerisation, which consists of an alteration in the internal constitution of the chlorophyll molecule. Unaltered chlorophyll on saponification with alkali gives a brown colour, which, after a few minutes, changes back to green. Allomerised chlorophyll does not give this "brown phase" on saponification.

(*c*) The phytol group may have been attacked. This is likely to take place if the chlorophyll is extracted from leaves rich in the enzyme chlorophyllase. The substances formed by such an action on the phytol group are most frequently the acid chlorophyllide, the presence of which may be detected by extraction of the ether solution with N/100 KOH, while the alkyl chlorophyllides, which possess strongly basic properties, are extracted with a 22-per-cent. HCl solution.

(*d*) A further proof that the chlorophyll has remained unaltered is that, on saponification with hot alkali, it shall give the dissociation products "phytochlorin *e*" and "phytorhodin *g*."

The Preparation and Properties of a Colloidal Solution of Chlorophyll.

Earlier investigators have used either a true solution of chlorophyll or a film of solid chlorophyll. A few investigators have used what they call an "emulsion," by which must presumably be understood water containing solid lumps of chlorophyll. In view of Willstätter's researches it is extremely unlikely that any solvent which dissolves chlorophyll is present in sufficient quantity in the leaf to give a true solution, nor is it at all likely that chlorophyll is present as a solid layer. There is good reason therefore to assume with Willstätter that chlorophyll is present in the leaf in the colloidal condition.

Chlorophyll in colloidal solution is an electronegative suspensoid. In the preparation of a chlorophyll sol a method is used which is frequently employed to obtain colloidal solutions, viz., the replacement of the solvent of a true solution of the substance by a medium in which the latter is insoluble, but which is miscible with the solvent. Thus chlorophyll is soluble in methyl alcohol, ethyl alcohol, acetone, and pyridin, but insoluble in water. A colloidal solution can therefore be prepared by dissolving chlorophyll in any one of these four solvents and mixing the solution so obtained with a large volume of water.

By using a colloidal solution with water as the dispersion medium one has a definite system which can be reproduced at will.

Further, in such a system, other substances (gaseous, liquid, or solid) can

be introduced so as to be uniformly distributed among the original components of the heterogeneous system.

It is important to bear in mind that actions in heterogeneous systems are not comparable with those taking place in true solutions.

Preparation.—The chlorophyll sol was prepared by dissolving 0·40 grm. of pure chlorophyll ("*a*"+"*b*") in 3 c.c. of absolute alcohol, and adding the solution to 300 c.c. of distilled water. This was the sol used in most of our experiments.

The way in which the alcohol solution is mixed with the water influences the dispersion, but by always making up the sol under exactly the same conditions, uniformity can be obtained.

Properties.—The chlorophyll sol is pure green in colour, somewhat opalescent, without fluorescence, and of a much greater depth of colour than a true solution of the same strength.

Chlorophyll sol offers opportunities for very exact investigations into the properties of suspensoids owing to the fact, first pointed out by Willstätter, that certain of the solvents of chlorophyll which are immiscible with water, such as ether, petrol-ether, benzene, do not remove chlorophyll from colloidal solution when shaken up with it, but immediately remove it if it is precipitated. Consequently the extent of precipitation of the colloid can always be estimated by colorimetric examination of the ethereal extract.

We found that in 24 hours chlorophyll was completely precipitated from the colloidal solution by sodium chloride when the latter was present in concentration N/25, but in N/30 sodium chloride no precipitation took place. Similarly with magnesium chloride a concentration of N/100 causes complete precipitation of the chlorophyll, while there is no precipitation when the concentration of the magnesium chloride is reduced to N/125. Addition of small quantities of alkalies stabilises the sol, while precipitation is facilitated by additions of small quantity of acid. Strong acids are particularly efficient in effecting precipitation. Thus the sol described above was precipitated in N/500 sulphuric acid. If, however, a weak acid of a much higher concentration, for example, N/5 boric acid, is used, no precipitation takes place.

If such a colloidal solution without any added substance is exposed to air there is no precipitation, but it will gradually change in colour to yellow-green and finally bleach, the rate at which this takes place being greater in the light than in the dark.

Owing to the method of preparation of the chlorophyll sol described above it will be observed that the colloidal solution contains a small quantity of solvent (*e.g.*, absolute alcohol) in which the chlorophyll is first dissolved. The influence of the presence of this solvent in any system used must obviously

be considered. From among the four substances at our disposal (methyl and ethyl alcohols, acetone, and pyridine) we chose ethyl alcohol, for the following reasons. Pyridine is the best solvent for chlorophyll and mixes very readily with water, but on account of its strongly basic properties it would be impossible to examine the influence of acids in its presence. As carbon dioxide forms only a weak acid with water, the action of this gas on a chlorophyll sol could not be examined in presence of pyridine. There is also a difficulty in obtaining pyridine absolutely free from impurities, and it gives oxidation products on exposure to light which interfere with tests for formaldehyde. Also owing to its high boiling point it cannot be removed by evaporation under reduced pressure.

The use of methyl alcohol is also inadvisable, as tests showed that under the conditions of our experiments it oxidises very readily to formaldehyde. Similarly acetone was not available, as it also produces formaldehyde on exposure to light. It must be emphasised that to the other sources of error in the experiments of earlier workers dealing with the production of formaldehyde by chlorophyll this one, arising from the presence of traces of the solvent used, must be added.

Ethyl alcohol on exposure to ultra-violet light in quartz vessels gives formaldehyde, but not when exposed in glass tubes. Therefore ethyl alcohol was used in the preparation of the chlorophyll sol and glass vessels were generally employed, quartz vessels being only used for special purposes. When necessary the concentration of alcohol in the colloidal solution was reduced by evaporation under reduced pressure.*

EXPERIMENTS.

In these experiments a definite quantity of the chlorophyll sol was introduced into test-tubes from which the air was subsequently evacuated. The tubes were then filled with the particular gas or gas mixture desired, sealed off, and exposed to light. When a mercury vapour lamp (3·5 amp.) was used, they were placed at a distance of 10 cm. from the lamp.

The results obtained with different gases were as follows:—

* In this connection we should like to draw attention to a simple method, much superior to the iodoform method in sensitiveness, for estimating small quantities of alcohol (Klöcker, 4). By this method one part of alcohol in 500,000 to 1,000,000 can be detected; 5 c.c. of the liquid to be examined are put in a tube 180 mm. long and 24 mm. in diameter, closed with a cork through which passes a glass tube 80 cm. long and 3 mm. in diameter, the lower part not prolonged below the lower level of the cork. The liquid is then boiled gently with the tube in a vertical position. If alcohol be present characteristic oily drops appear in the tube; the less alcohol present the higher the level in the tube at which the drops appear. The same reaction is given with acetone.

(a) *Nitrogen.*

On exposure of chlorophyll sol to light in presence of nitrogen no apparent change takes place to the chromogen complex of the chlorophyll molecule, even after several hundred hours' exposure to the light of a mercury vapour lamp, and no formaldehyde is produced.

(b) *Carbon Dioxide.*

Colloidal solutions of chlorophyll of different concentrations were exposed to light in presence of carbon dioxide. In all cases there was a rapid change of the pigment to yellow-green or brownish-green, the colour change being preceded in the sols of highest concentrations by precipitation of the colloid. The same change takes place if the tubes are kept in the dark, but the action is considerably slower. The colour is not further changed by prolonged exposure to light, nor by any further keeping in the dark.

It may be recalled here that the action of acids on chlorophyll is to produce the magnesium-free derivative phæophytin, which possesses this same yellow-green or brownish-green colour. As carbon dioxide in water forms a weak acid, it at once suggests that the action of carbon dioxide on a colloidal solution of chlorophyll is simply that of an acid. That this is indeed the explanation of the change observed in the chromogen complex of chlorophyll becomes evident from the following experiments:—

1. An exactly similar colour change is produced when the chlorophyll sol is treated with other weak acids, such as boric acid or acetic acid. If, for instance, some chlorophyll sol is sealed off in an atmosphere of nitrogen with boric acid (for instance, 5 c.c. of sol + 1 c.c. N. boric acid) and exposed to light, the colour change is exactly similar to that in the tube containing the chlorophyll sol and carbon dioxide, and takes place in about the same time.

2. If the sol is made up in water from a solution of chlorophyll in pyridine, no change in colour takes place in an atmosphere of carbon dioxide. The sol contains a little pyridine, which is a strong base, so the acid is neutralised and the change to phæophytin prevented.

If a sol made up from an alcohol solution is kept in an ordinary loosely stoppered bottle in the dark it will, in the course of a few months, gradually turn yellow-green. A sol made up from a pyridine solution will under the same conditions remain quite unchanged in colour.

3. It is possible to maintain approximate neutrality in experiments with carbon dioxide by using sodium bicarbonate in the solution. A solution containing H_2CO_3 and $NaHCO_3$ in the proportions of 1 to 3·75 is approximately neutral, but to avoid precipitation of the colloid the concentration of the bicarbonate must be kept low, consequently full atmospheric pressure

of carbon dioxide cannot be employed. Accordingly a pressure of 380 mm. CO_2 + 380 mm. N_2 was used. In such a system the colour of the chlorophyll remained unchanged.

4. The yellow-green derivative, produced by the action of carbon dioxide on chlorophyll, was precipitated by magnesium chloride and extracted with ether. The ethereal extract was evaporated to dryness and the pigment dissolved in alcohol. On addition of a trace of copper acetate to the boiling alcohol solution a bright green colour appears. This reaction is characteristic of the magnesium-free derivatives of chlorophyll. When the magnesium of the chlorophyll molecule is replaced by hydrogen in the formation of phæophytin this is accompanied by the colour change from green to yellow or brownish-green already noted. On treating phæophytin with copper acetate the hydrogen is replaced by copper, and with the re-entrance of a metal (copper) into the chromogen complex, the colour changes back to a brilliant green. This colour change is very striking. A similar green compound is obtained when zinc acetate is used.

The compound obtained with boric acid behaves in a similar way towards copper acetate.

5. The yellow-green substance in ethereal and alcoholic solution was submitted to a careful spectroscopic examination. It gave the characteristic absorption spectrum of phæophytin, chiefly characterised by a strong absorption band before the line E and another one between the lines E and F.

The substance obtained with boric acid gave an identical spectrum.

The experiments described under 4 and 5 were also carried out with a true solution of chlorophyll in alcohol (about 98 per cent.) which was subjected to the action of carbon dioxide; identical results were obtained. A much greater amount of carbon dioxide is necessary here, owing probably to the lesser degree of ionisation of the carbonic acid in alcohol.

Formaldehyde Test.—Tests for formaldehyde were made after short and long exposures of the colloidal solution in the presence of carbon dioxide and also with the bicarbonate system. Various sources of illumination were used, sunlight, arc-lamp, mercury-vapour lamp, but in no case was any indication obtained of the presence of formaldehyde.

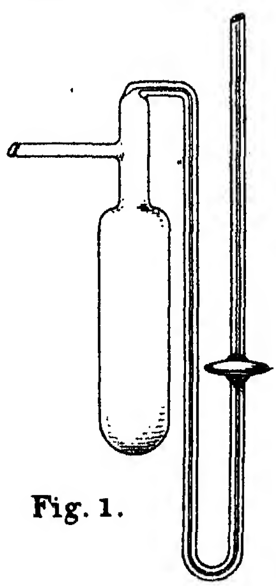


Fig. 1.

We have attempted, by means of the apparatus figured (fig. 1), to

determine whether the illuminated chlorophyll sol has any action on carbon dioxide. If any disappearance of carbon dioxide, due to a catalytic action of chlorophyll, takes place under the conditions, it might be possible, even with small quantities of chlorophyll, to get appreciable diminution of the carbon dioxide present.*

The apparatus consists of a glass vessel provided with a side tube and a manometer with a tap. The procedure is as follows: the chlorophyll sol is introduced through the side tube, the vessel is evacuated, carbon dioxide introduced, and the side tube sealed off. The apparatus is placed in a thermostat, and, by noting the barometric pressure, the pressure of the gas can be accurately determined. An apparatus of this description enables one to follow from time to time changes in the gas-content of the vessel.

If a strong concentration of the sol is used precipitation takes place. Hence, only the results of experiments with moderate concentrations of the sol are here described. Strong concentrations up to 200 mgrm. in 100 c.c. are employed, but the question of absorption here becomes so complex that a detailed investigation is required.† With moderate concentrations no appreciable absorption was found, although in most cases an absorption of 0·001 grm. of carbon dioxide could have been detected. The following is a typical result: 20 c.c. of a sol (concentration 40 mgrm. in 300 c.c. water) were contained in the vessel of volume 120 c.c., filled with carbon dioxide under a pressure of 820 mm. of mercury, at a temperature of 20° C. The pressure of the gas remained unchanged for 24 hours. The following Table indicates the colour change taking place in the experiment.

Time of illuminations.	Pressure.	Colour.
hr.	mm. Hg.	
0	821	Green.
4	821	Yellowish green.
5	820	" "
8	822	Yellow brown.
10	819	No further change.
20	820	" "

The slight variations in pressure are within the limits of experimental error.

* It is possible, on the other hand, that catalysis, though occurring, might not proceed to any appreciable degree *in vitro*, while in the living plant it might nevertheless proceed vigorously owing to a continual removal of products.

† It is clear three processes are possible here: (1) absorption of carbon dioxide by the dispersion medium, (2) absorption of carbon dioxide by the chlorophyll phase, (3) removal of carbon dioxide by chemical action during illumination. The method here employed would give information concerning the last process only.

At the end of the experiment the gas was analysed in a Haldane gas analysis apparatus, and was found to consist of 100 per cent. carbon dioxide.

(c) *Oxygen.*

It is well known that chlorophyll bleaches rapidly in air or in oxygen when exposed to light. This was also the case in our own experiments with a colloidal solution of chlorophyll. The bleaching is preceded by a change in colour from green to yellow-green or brown-green, similar to that produced when carbon dioxide was used. Spectroscopic examination indicates the presence of phæophytin. It seems reasonable to suppose that an acid substance is produced during the bleaching, which reacts with the chlorophyll not yet bleached, giving phæophytin in the usual way. If a small quantity of alkali is added the brown-coloured stage is omitted and bleaching accelerated.

Formaldehyde Test.—Formaldehyde is not produced in any quantity during the bleaching of chlorophyll in oxygen, but, after bleaching is complete, formaldehyde is formed in amounts which can be easily estimated. We have made estimations of the quantity of formaldehyde produced by the action of oxygen on chlorophyll after various times of exposure to light, using Schryver's method for estimating small quantities (Schryver, 6, 7). The results of these experiments are summarised in the accompanying Table, and are exhibited graphically in the curves shown in fig. 2.

We have already mentioned the production of formaldehyde from alcohol exposed in quartz vessels. The curve C shows how large is the quantity of formaldehyde produced when this substance is present. In this case a chlorophyll sol (with traces of alcohol) was exposed to light in a large quartz vessel, and samples examined from time to time. In the case of glass vessels, the presence of traces of ethyl alcohol makes no difference in the amount of formaldehyde produced, as is shown by a comparison of curves A and D.

The small quantities of formaldehyde obtained before bleaching is complete are no doubt produced from the part of the chlorophyll already bleached.

It seems reasonable to suggest that the production of formaldehyde is due to the oxidation of the methyl or phytyl ester groups of the chlorophyll molecule. According to Willstätter, the alcohol phytol is a primary alcohol of formula $C_{20}H_{39}OH$, whose structure may be represented thus:—

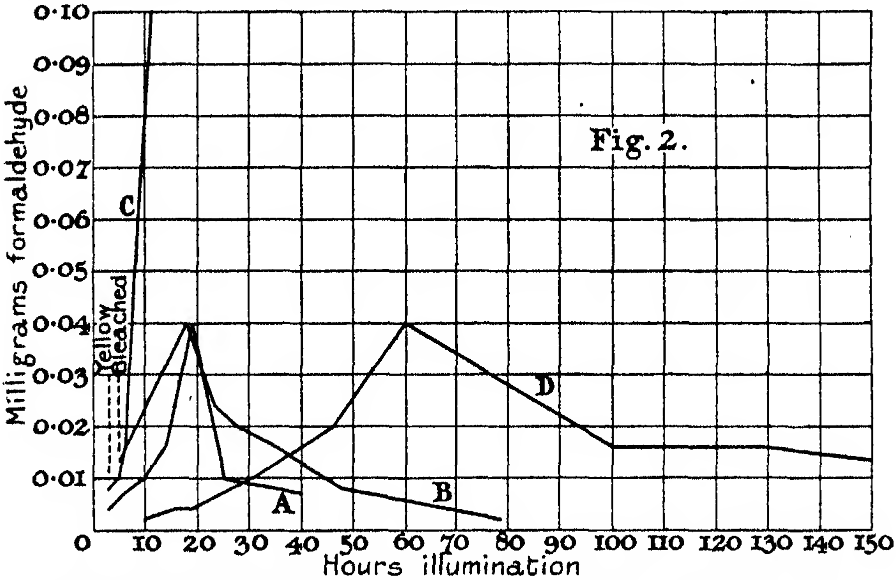
$$CH_3-\underset{CH_3}{\underset{|}{CH}}-\underset{CH_3}{\underset{|}{CH}}-\underset{CH_3}{\underset{|}{CH}}-\underset{CH_3}{\underset{|}{CH}}-\underset{CH_3}{\underset{|}{CH}}-\underset{CH_3}{\underset{|}{CH}}-\underset{CH_3}{\underset{|}{CH}}-\underset{CH_3}{\underset{|}{C}}=\underset{CH_3}{\underset{|}{C}}-CH_2OH$$

In the oxidation and breaking down of this alcohol, it is evident that

Table II.

Milligrammes of chlorophyll originally present.	Milligrammes formaldehyde present after given intervals.											Remarks.
0·6	Hours	3	6	10	14	19	24	39				Thin glass tubes, 100 per cent. O_2. Each estimation made from a separate tube. Traces of ethyl alcohol not removed.
	Mgrm. formaldehyde ...	0·004	0·007 (bleached)	0·01	0·016	0·04	0·01	0·007				
0·5	Hours	5	18	23	28	35	48	78				Quartz vessel, 100 per cent. O_2. Traces of alcohol removed. Estimations made by withdrawing samples at intervals.
	Mgrm. formaldehyde ...	0·013	0·04 (bleached)	0·024	0·02	0·016	0·008	0·002				
0·5	Hours	3	4½	6	10	16	20	40				Quartz vessel, 100 per cent. O_2. Traces of alcohol not removed. Estimations made by withdrawing samples at intervals.
	Mgrm. formaldehyde ...	0·008	0·01	0·016	0·08	0·4	1·2	1·8				
0·6	Hours	10	16	19	27	31	46	60	100	130	200	Thick glass tubes, 100 per cent. O_2. Each estimation made from a separate tube. Traces of alcohol not removed.
	Mgrm. formaldehyde ...	0·002	0·004 (bleached)	0·004	0·008	0·01	0·02	0·04	0·016	0·016	0·01	

formaldehyde might be produced in large quantities. This does not exclude the possibility that the complete breaking down of the chromogen complex may also yield formaldehyde among its oxidation products.



Curves of Formaldehyde Production in the Photo-oxidation of Chlorophyll Sols.

These curves are based on the data given in Table II. In the case of curves A and D glass tubes were used, but the glass was thick in D, thin in A; also the traces of ethyl alcohol were removed in the case of D, but not in the case of A. In the case of curves B and C a quartz vessel was used; the alcohol was removed in the case of B, but not in the case of C.

It will be observed from the curve (fig. 2) that the quantity of formaldehyde produced reaches a maximum, and then decreases in amount. This is probably due to further oxidation of the formaldehyde to formic acid. The liquid, indeed, increases in acidity from the beginning of the experiment.

Criticism of Previous Theories of the Function of Chlorophyll in Carbon Assimilation in view of the Experimental Data obtained in this Investigation.

a. *Theory of Usher and Priestley.*

In a series of papers (10) on the mechanism of carbon assimilation, these authors have expressed the opinion that the initial stages of this process are "entirely non-vital and can be reconstructed *in vitro*." They consider the first stage as consisting of the photolysis of aqueous carbonic acid with the production of formaldehyde and hydrogen peroxide, the evolution of oxygen being due to the decomposition of the latter substance by catalase.

This conclusion is in strong contrast to that drawn by Willstätter from his own experiments (14, 15). "In spite of numerous experiments with extracted chlorophyll or isolated chloroplasts we have been unable to reproduce the phenomena of carbon assimilation outside the plant." Unfortunately this important statement was not accompanied by any experimental evidence. It is, however, the view to which the present authors have been led.

We do not intend to deal with the indirect evidence brought forward by Usher and Priestley in support of their theory, such as the production of formaldehyde from carbon dioxide and water exposed to light in quartz tubes; these and similar experiments by other investigators have been criticised adversely by H. A. Spoehr (8).

With regard to the direct evidence of the production of formaldehyde in chlorophyll-containing systems, we have not been able to obtain any evidence of formaldehyde production in systems containing chlorophyll, carbon dioxide, and water. Formaldehyde production was always limited to systems containing oxygen. But even minute traces of oxygen are capable of accounting for small quantities of formaldehyde. If crude chlorophyll is used the small amount of oxygen present in the commercial nitrogen and carbon dioxide obtained from gas cylinders can give rise to a large formaldehyde production.

Although some of Usher and Priestley's results can be explained by the oxidation of crude chlorophyll, other of the results can hardly be interpreted as due to this cause. Thus these authors give an account of some experiments (1911, p. 103), of which they state that they carried out a considerable number with concordant results. In these experiments there were exposed to light two pairs of sealed glass tubes containing:

(1) Chlorophyll, air, catalase, and caustic potash, (2) chlorophyll, air, and caustic potash; (3) chlorophyll, air, catalase, and caustic potash, (4) chlorophyll, catalase, air, and carbon dioxide.

They found that the chlorophyll bleached much more quickly in tubes 2 and 4 than that in 1 and 3. While 3 gave only a trace of formaldehyde, 4 exposed to light for the same time gave a marked formaldehyde reaction.

It is indeed tempting, and seems at first sight quite reasonable, to assume that the increased quantity of formaldehyde produced in 4 as compared with 3 is due to the carbon dioxide, and that 1 remains green longer than 2 because in the former the catalase destroys the hydrogen peroxide responsible for the bleaching.

But considered more closely these experiments become quite concordant with our results. Usher and Priestley have neglected the possibility of the

activity of the enzyme being affected by the different experimental conditions in the various tubes. Sörensen (9), however, has emphasised the fact that for many enzyme actions there is not only a temperature optimum but also a definite concentration of hydrogen ions at which maximum activity is obtained. For catalase Sörensen found that the optimum activity was obtained at absolute neutrality, diminishing with increase both of acidity and of alkalinity. In Usher and Priestley's experiments the catalase was mixed with gelatine, into which carbon dioxide would diffuse, and the increased acidity resulting would inhibit the action of the catalase.

The result of Usher and Priestley's experiments would then be explained thus:

1. The production of formaldehyde is always due to the oxidation of chlorophyll or the accompanying substances in the crude chlorophyll.

2. The bleaching is due to the oxidation of chlorophyll under the action of light.

3. The inhibition of bleaching in the tube 1 in the experiment referred to above is due to the catalase system preventing the oxygen from acting on the chlorophyll.

4. The formaldehyde production in tube 4 is due to the decrease in the activity of the catalase system owing to the acidity produced by carbon dioxide, formaldehyde being produced by oxidation of the chlorophyll. Unfortunately tube 2 was not examined for formaldehyde.

b. *Theory of Wager.*

Wager (11) observed an active absorption of oxygen by his chlorophyll extract and the formation, as the result of oxidation, of considerable quantities of formaldehyde. On these two observations Wager suggests a theory of carbon assimilation in which carbon dioxide is in one way or another "built up independently into the chlorophyll molecule," and the carbohydrate production then "initiated by the photo-oxidation of chlorophyll and subsequent polymerisation of the aldehyde thus formed, rather than by the direct photo-synthesis of carbon dioxide and water."

Neither of the two observations on which the theory is based can be considered satisfactory. It has been shown by Willstätter that it is only the yellow pigments which are able to absorb oxygen with rapidity and in large quantities. As Wager states that he worked with a crude alcoholic extract of the leaf pigments, evaporated to dryness and redissolved in petrol-ether, the rapid absorption of oxygen observed by him must almost certainly have been due to the yellow pigments, and cannot therefore be used in support of a theory of the action of chlorophyll.

As to the production of an aldehyde on oxidation, it has been shown above that, as far as chlorophyll is concerned, formaldehyde is only produced at a late stage in the process of oxidation after bleaching of the chlorophyll has taken place. It is thus a purely secondary product, arising probably from the phytol which has been split off from the chlorophyll molecule.

c. *Theory of Ewart.*

It is obvious from Ewart's paper (1) that he was not acquainted with the work described in Willstätter's book (13), and it is clear that the method of extraction Ewart employs, which he calls a modification of Willstätter's, will not give pure chlorophyll. It is significant also from the description of his "pure" pigments that they have properties different from those of Willstätter, for instance, with regard to solubility. Also the formula for chlorophyll given by Ewart is that suggested by Willstätter in earlier papers, and is not the one put forward by Willstätter in his later book.

Ewart is one of the first among recent workers to realise that carbon dioxide assimilation is not a simple process, the first stage of which is represented by the equation $CO_2 + H_2O = HCHO + O_2$, but a complex one in which at least two pigments and their derivatives take part. However, it is clear that the theory advanced by Ewart is not sufficiently supported by experimental evidence. His main hypothesis is that carbon dioxide and water combine with the phytyl group to give xanthophyll and oxygen. A portion of this oxygen is then used to oxidise the xanthophyll into phytol, hexose sugars and formaldehyde, while the remainder is excreted from the chloroplast. The formaldehyde is then polymerised to hexoses either by contact with the chlorophyll or by dilute alkali in the protoplasm around the chloroplast.

Without entering into any detailed discussion of Ewart's hypothesis attention must be drawn to the fact that in our experiments we have never been able to obtain xanthophyll in a system containing chlorophyll, carbon dioxide, and water, but we have obtained a yellowish-green substance which we have identified as phæophytin. One is almost forced to assume either that Ewart's chlorophyll contained xanthophyll, or that the yellow substance he obtained was phæophytin. Unfortunately Ewart does not state on what evidence he relied for the identification of his xanthophyll.

In regard to Ewart's hypothesis of the re-formation of chlorophyll from xanthophyll it has to be remembered that the magnesium-free derivatives of chlorophyll very easily take up certain other metals and form a colour complex resembling that of chlorophyll; even the small traces of metals contained in the walls of glass vessels are sufficient for this. Thus Willstätter was able to test for the presence or absence of zinc in glass

vessels by the zinc chlorophyll formed from a magnesium-free derivative of chlorophyll. It suggests that Ewart's experiment on the re-formation of chlorophyll from a mixture of xanthophyll and bleached chlorophyll (in one case indeed zinc dust was added) should be interpreted as the taking up of a metal by a yellowish or colourless magnesium-free derivative, by which a complex, chlorophyll-green in colour, is produced.

Conclusion.

The result of this investigation is to show how unsatisfactory is the experimental evidence on which the various theories of the function of chlorophyll are based.

Summary.

1. Certain photochemical reactions of chlorophyll, on which previous workers have based theories of carbon assimilation, have been examined. Earlier investigators have used crude chlorophyll containing, besides the two chlorophyll pigments, yellow pigments and other substances. In the present experiments pure chlorophyll ("*a*"+"*b*"), extracted by the method of Willstätter and Stoll, and satisfying the criteria of purity given by them, has been used.

2. The chlorophyll was used as a sol, with water as dispersion medium. This sol, in contact with various gases in closed vessels, was exposed to light. The following are the results obtained:—

(a) *Nitrogen.*—In nitrogen no change in the chromogen complex of the chlorophyll molecule takes place.

(b) *Carbon Dioxide.*—In carbon dioxide the magnesium-free derivative phæophytin is produced. In this action carbon dioxide behaves in solution like any other weak acid. No further change in the chromogen complex takes place. Formaldehyde is not formed.

(c) *Oxygen.*—In oxygen the first change observed is the yellowing and ultimate bleaching of the chlorophyll. The yellowing is due to the presence of phæophytin. If alkali is added this yellow stage is omitted and the bleaching accelerated. In the first stages, while bleaching is in progress, formaldehyde is produced only in very small quantities, but, after bleaching is complete, the quantity of formaldehyde increases, rapidly reaching a maximum and then diminishing. The acidity of the system, on the other hand, increases throughout. It is suggested that the formaldehyde arises mainly from the phytol, which is probably split off from the chlorophyll molecule under the action of light and oxygen.

3. The hypotheses put forward by Usher and Priestley, H. Wager, and

Ewart, as to the chemical changes occurring in the process of carbon assimilation by green plants, are not supported by experimental evidence.

In conclusion, we would thank Mr. W. Stiles for his co-operation in the work of extraction and purification of the pigments, and we should like to express our indebtedness to Prof. V. H. Blackman for the facilities he has given us, and for his continued advice and criticism.

[*Note added October* 12, 1916.—Of Willstätter's three papers on chlorophyll which appeared in 1915, only that in the 'Berichte der deutschen chemischen Gesellschaft' (1915, No. 13, p. 1540) was available at the time the manuscript of this paper was completed; the other two ('Sitzungsber. d. kgl. preuss. Akad. der Wissen.,' 1915, p. 322 and p. 544) were only accessible in the form of very brief abstracts. We were thus unaware that Willstätter had made experiments on the behaviour of a chlorophyll solution exposed to carbon dioxide. Now that copies of the journal containing the other two papers are available in this country, we find that Willstätter has made such experiments, and arrives at the conclusion put forward above, that phæophytin is produced under these conditions.]

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Investigations dealing with the Phenomena of "Clot" Formations. Part IV.—*The Diphasic* Erosive Action of Salts on the Cholate Gel.*

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(Communicated by V. H. Blackman, F.R.S. Received June 26, 1916.)

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Introduction.

In the last communication† of this series, attention was called to the fact that the cholate gel is eroded when immersed in solutions of sodium chloride and other chlorides, and that this erosive action can be "antagonised" by addition to the chloride solutions of relatively small amounts of calcium chloride. The analogy between this action and the biological "antagonistic" action of calcium towards sodium and other salts was also commented upon and illustrated by examples from the researches of Loeb, Osterhout and other investigators.

In addition, however, to the antagonism between calcium salts on the one hand and sodium, potassium and magnesium salts on the other hand, Loeb has shown in the case of *Fundulus* that there is evidence of antagonism between potassium and sodium salts, that is to say that the toxic action of one salt can be rendered more or less innocuous by the addition of the other. Osterhout has shown that certain marine plants will retain their vital

* The term "diphasic" used in the sub-title to this paper refers to the diphasic form of the curve produced when the concentrations of the eroding salts are plotted as abscissæ and the amounts of erosion as ordinates.

† Schryver, 'Roy. Soc. Proc.,' B, vol. 89, p. 176 (1916).

activities for a longer period in a solution which contains, in addition to sodium and calcium salts, also potassium and magnesium salts, and has formulated the conception of "balanced" salt solutions as necessities for the maintenance of the maximal activities of plants.

The investigations on the salt actions already published have, so far, revealed only the analogy between biological antagonism of calcium to certain other salts, and an antagonistic action of a similar nature on the cholate gel. In view of the conception advanced by one of the authors of this paper, that the protoplasm or cell-membrane contains a gel-forming substance alike in many of its physical properties to cholate gels, it was of interest to determine whether further analogies exist between the biological action of salts and their erosive action on the cholate gel—to ascertain, for example, whether mixtures other than those of calcium chloride and one of the chlorides of alkali metals could form "balanced" solutions in which the gel can maintain its stability.

Now as the hypothesis has been put forward that plant and animal cells contain gel-like structures, and that their normal activities are intimately connected with the maintenance of a certain state of aggregation of such structures, it was of importance to study the action of salts on gels which might simulate as closely as possible those postulated for in the living cell. These should contain, in addition to the substance to which the gel structure is primarily due, certain salts and other bodies to which must be ascribed the relatively high osmotic pressure of the cell. Now in all the experiments already carried out on the erosion of the cholate gel, the material employed has been relatively poor in inorganic salts; the solution from which the gel was prepared by heating contained, in fact, 2 per cent. sodium cholate (the concentration of which was less than N/20) and calcium chloride in the concentration of N/40 (M/80). In the present communication are contained the results of a series of experiments on the action of salts on gels containing larger amounts of added salts. The results indicate that gel stability in the presence of salts is determined by the action of several factors. Much research remains to be accomplished before the laws governing gel formation and disaggregation can be elucidated and the biological actions of salts explained.

Experimental.

The experimental method employed was the same as that described in the last communication.*

* *Loc. cit.*, p. 176.

Four gels were prepared from the following series of mixtures :—

2 c.c. 4-per-cent. sodium cholate + 0·5 c.c. 3 N/10 $CaCl_2$ + 1·5 c.c. N/3 LiCl.
" " " " " 1·5 c.c. N/3 NaCl.
" " " " " 1·5 c.c. N/3 KCl.
" " " " " 1·5 c.c. N/3 $MgCl_2$.

The concentration of calcium chloride in the mixtures, therefore, was 3N/80 and of the added salts N/8. The mixtures were introduced into U-tubes of about 3 mm. internal diameter, and clotted by heating for ½ hour in a thermostat at 50°. The tubes were then removed from the thermostat, and cut into lengths of 75 mm., which were introduced into a series of Petri dishes containing 20 c.c. of the different salt solutions of varying concentrations. After 17 hours, the tubes of gel were removed and the amount of erosion was measured. The experiments were carried out at room temperatures. *The numbers given in Table I, which represent the amount of erosion in millimetres, are not strictly comparable, as the experiments of each gel were carried out at different times and at slightly different temperatures. All the experiments with each individual gel were, however, carried out at the same time.*

Table I.—Erosion of Gels (in millimetres) containing Different Salts (in Concentrations N/8) when Immersed in Eroding Salt Solutions of Varying Concentrations.

Salt added to gel.	Salt in eroding solution.	Concentrations of the salt in eroding solutions.									
		N/20.	2N/20.	3N/20.	4N/20.	5N/20.	6N/20.	7N/20.	8N/20.	9N/20.	10N/20.
LiCl	LiCl ...	15	39	56	68	46	28	17	20	21	26
	NaCl ...	14	24	38	19	18	25	34	50	70	>75
	KCl ...	16	19	21	17	20	27	38	52	72	>75
	$MgCl_2$ ...	12	36	31	22	16	22	30	37	42	46
NaCl	LiCl ...	15	43	64	69	69	61	57	26	19	20
	NaCl ...	15	38	58	44	19	19	22	34	48	66
	KCl ...	18	18	32	22	16	14	22	32	48	55
	$MgCl_2$ ...	11	34	34	34	26	14	20	28	38	38
KCl	LiCl ...	16	45	62	78	72	66	[illegible]	51	18	20
	NaCl ...	14	31	52	50	28	18	22	35	48	56
	KCl ...	19	20	24	24	18	19	25	35	50	65
	$MgCl_2$ ...	10	29	30	35	27	18	20	29	35	40
$MgCl_2$	LiCl ...	44	78	75	75	78	58	32	28	23	17
	NaCl ...	30	50	49	30	16	18	20	32	47	62
	KCl ...	35	40	32	20	16	18	23	35	45	64
	$MgCl_2$ ...	42	50	50	45	18	16	20	32	45	50

The amount of erosion of the various gels in water lay between 12 and 14 mm.

The results of the erosive action of different salts on the gel containing added potassium chloride are shown graphically in fig. 1, where the concentrations of the eroding solutions are plotted as abscissæ and the amounts of erosion as ordinates. The curves obtained with gels containing other added salts do not differ materially from this one.

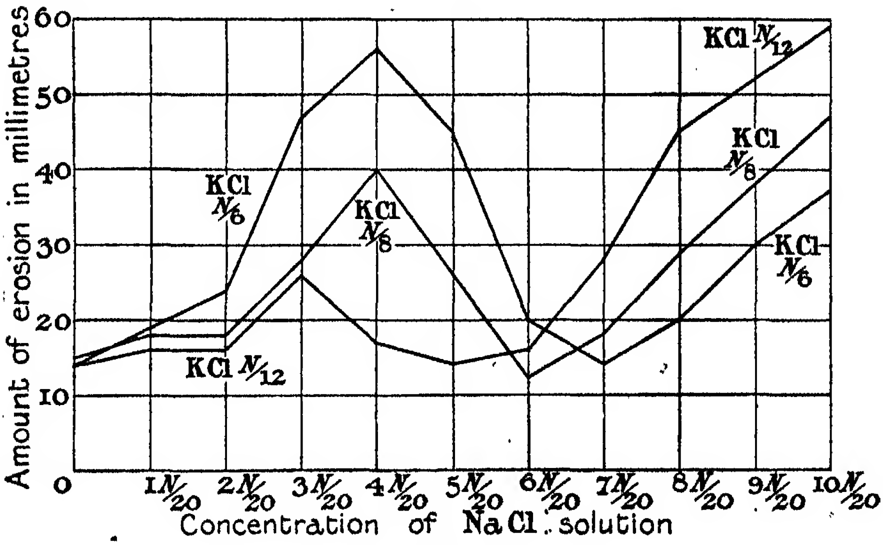

Fig. 1.

It will be noticed that the curves possess a diphasic character, with two minima and two maxima. The amount of erosion increases with increase in the concentration of salt in the eroding solution up to a certain point (3N/20—4N/20). On increasing the concentration beyond this, the amount of erosion diminishes, until it reaches a minimum (6N/20 in the case of the chlorides of potassium, magnesium and sodium, and 9N/20 in the case of lithium chloride), when it is little more than that which takes place in the presence of pure water. On increasing the concentration above this point the erosion again increases. Results of this character have been obtained constantly; only a portion of them are recorded in this paper.

There is a considerable variation in the effects produced by the various salts in the eroding solution. The maximal eroding effect produced by the lower concentrations, shown both by the amount of erosion and the breadth of the "zone of instability" (as it is convenient to designate the interval between the two minima of the curve), is produced by the lithium salt, followed, in decreasing order of action, by the chlorides of sodium, magnesium and potassium. It has been found by Stiles and Jörgensen* that the effect

* 'Annals of Botany,' vol. 29, p. 349 (1915).

of the action of chlorides on the permeability of certain vegetable tissues follows in the same order.

The breadth of the zone of instability is also affected by the concentration of the salts contained within the gel. When the gel is made by heating a solution containing 2-per-cent. sodium cholate and 3N/80 calcium chloride, with no other added salt, the diphasic character of the curve is only marked in the cases where lithium and magnesium chlorides are in the eroding fluid; in both these cases two maxima in the curve can be observed; in the cases of sodium and potassium chlorides only faint indications of a diphasic character are obtained.* The experimental results are given in Table II.

Table II.—Erosive Action (measured in mm.) of Salts on Gel containing no Added Salt. Gel 2 c.c. 4-per-cent. Sodium Cholate + 0·5 c.c. 3N/10 $CaCl_2$ + 1·5 c.c. H_2O.

	Concentrations of salt solutions.										
	0.	N/20.	2N/20.	3N/20.	4N/20.	5N/20.	6N/20.	7N/20.	8N/20.	9N/20.	10N/20.
LiCl	12	13	18	22	20	15	16	23	30	38	60
NaCl	—	12	6	12	18	26	42	54	73	>75	>75
KCl	—	10	7	9	14	26	85	47	66	67	71
$MgCl_2$ ...	—	9	19	11	15	27	42	51	52	55	61

Both the breadth of the "zone of instability" and the amount of erosion within this zone can be increased, however, by increasing the concentration of the salts within the gel. The results illustrating this statement are given in Table III, and plotted graphically in fig. 2. In these experiments the gels were made by heating the following solutions:—

2 c.c. 4-per-cent. sodium cholate + 0·5 c.c. 3N/10 $CaCl_2$ + 1·5 c.c. 2N/9 KCl.
" " " " " 1·5 c.c. 3N/9 KCl.
" " " " " 1·5 c.c. 4N/9 KCl.

The concentration of potassium chloride in these gels was, therefore, N/12, N/8, and N/6. The eroding solution used was that of sodium chloride.

The influence of certain non-electrolytes on gels was also investigated. Dextrose solutions have practically no erosive action on the gels, whether they contain added salts or not. On the other hand, the erosive action of salt solutions on gels containing dextrose is similar to that on gels containing

* The curves are not reproduced in this paper.

salts, and the curves representing the action give indications of the same diphasic character. The action of sodium chloride solution on gels

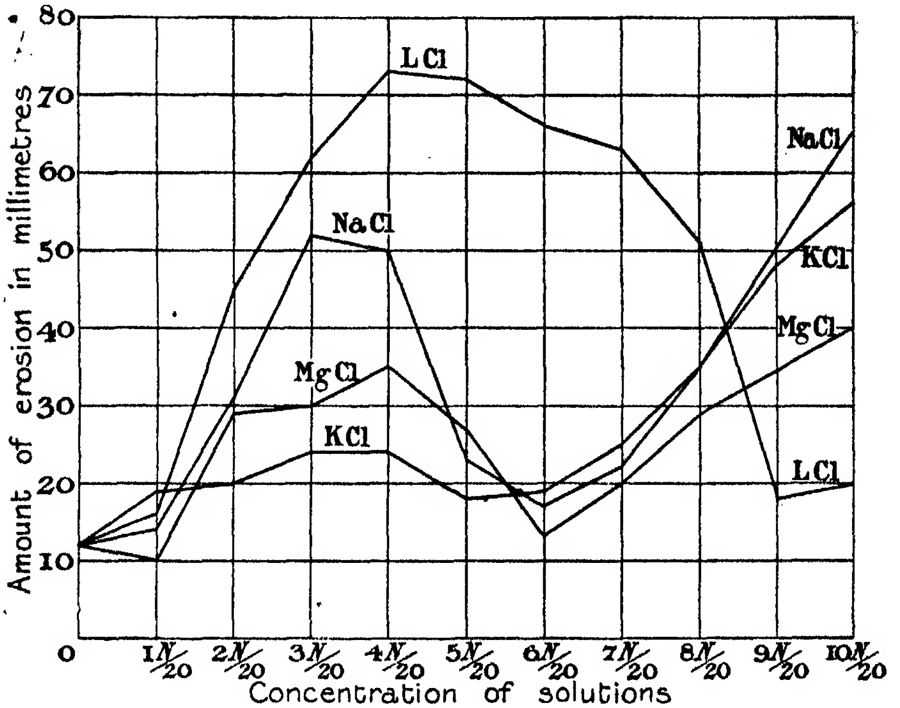

FIG. 2.

Table III.—Erosive Action (measured in mm.) of Sodium Chloride Solutions on Cholate Gel containing Varying Concentrations of Potassium Chloride.

Concentration of KCl in gel.	Concentration of NaCl solutions.									
	N/20.	2N/20.	3N/20.	4N/20.	5N/20.	6N/20.	7N/20.	8N/20.	9N/20.	10N/20.
N/12 ...	16	16	27	17	14	16	28	44	52	59
N/8	18	18	28	40	26	12	18	28	38	47
N/6	19	24	47	56	44	20	14	20	30	37

Erosion in water 14.

containing dextrose is illustrated by Table IV. The gels were made from the following mixtures :—

2 c.c. 4-per-cent. sodium cholate + 0·5 c.c. 3 N/10 $CaCl_2$ + 1·5 c.c. N dextrose.

" " " " " 1·5 c.c. 3N/9 "

The concentration of dextrose in the gels was, therefore, 3N/8 and N/8.

Table IV.—Erosion of Gels (measured in mm.) containing Dextrose by Sodium Chloride Solutions.

Concentration of dextrose in gel.	Concentration of sodium chloride.										
	0.	N/20.	2N/20.	3N/20.	4N/20.	5N/20.	6N/20.	7N/20.	8N/20.	9N/20.	10N/20.
3N/8	13	14	18	37	49	49	51	69	57	41	40
N/8	13	22	—	35	31	24	20	20	20	21	24

Antagonistic Action of Calcium Chloride to Sodium Chloride.

Experiments were undertaken to ascertain the amount of calcium chloride necessary to antagonise the erosive action of sodium chloride solutions of various concentrations. It will be observed from Table V, in which the results of these experiments are recorded, that the erosion produced in a gel (containing N/8 KCl) by a concentration of 3N/20 sodium chloride is about equal to that produced by the concentration 8N/20, when no calcium chloride is added to the eroding solutions. It was found that somewhat higher concentrations of calcium chloride were necessary to produce complete protective action against the 8N/20 solution than were required in the case of the 3N/20 solution of sodium chloride. Nevertheless, the ratio of calcium salt to sodium salt necessary to inhibit erosion within the "zone of instability" was larger than that which was required to produce complete antagonism in the region of the second phase of the erosion curve.

Table V.—Antagonistic Action of $CaCl_2$ to various Concentrations of NaCl. Gel. 2 c.c. 4-per-cent. sodium cholate + 0·5 c.c. 3N/10 $CaCl_2$ + 1·5 c.c. N/3 KCl.

Concentration of NaCl.	Amounts of erosion (in millimetres).				
	In absence of $CaCl_2$.	In presence of N/124 $CaCl_2$.	In presence of N/64 $CaCl_2$.	In presence of N/32 $CaCl_2$.	In presence of N/16 $CaCl_2$.
2N/20	18	6	5	3	0
3N/20	37	6	4	3	0
4N/20	44	7	4	2	0
5N/20	21	6	4	3	0
6N/20	14	7	5	2	0
7N/20	17	12	4	3	0
8N/20	35	22	5	4	0
9N/20	39	26	8	4	0

Finally, the action of various sodium salts (organic or inorganic) on cholate gels was investigated. When the gel was made in the ordinary

manner without added salts, the diphasic erosion curves were not obtained, and, generally, the solutions with low surface tensions had a greater erosive action than those with higher tensions. When the gel contained an added salt, however, a diphasic curve was always obtained, but the breadth of the "zone of instability" and the amount of erosion within this zone varied from salt to salt. In the second phase of the curve the solutions of low surface tensions exerted the greatest erosive action. Of particular interest is the action of sodium lactate on the gel containing potassium chloride (N/8), which shows a very narrow "zone of stability," but a very active erosion within this zone. The results are given in Table VI.

Table VI.—Erosive Action of Sodium Lactate on Cholate Gels.

	Concentration of Lactate.							
	N/20.	2N/20.	3N/20.	4N/20.	5N/20.	6N/20.	7N/20.	8N/20.
Gel without added salt	11	15	40	>75	>75	>75	>75	>75
Gel containing N/8 KCl	17	>75	>75	21	33	>75	>75	>75

It will be noticed that the erosion is very large in the concentration 2N/20—3N/20, but small in that of 3N/20—4N/20.

The salt solutions in all these experiments were made by neutralising exactly to phenolphthalein 2N solutions of sodium hydroxide with the acid, and then diluting with water till the concentrations were normal.

Theory of the Action of Salts on Gels.

The results recorded indicate that the action of salts on a gel system is a complex one, and that the stability is in all probability the resultant of several different factors acting simultaneously. In the following pages an attempt is made to summarise these. Certain of the generalisations will refer more especially to the cholate gel, whereas others will apply to gel structures in general.

The phenomena accompanying the formation of the cholate gel have been described in a former paper,* and these indicate that the gel itself consists of two phases, viz., a cholate-poor phase (designated hereafter as the "aqueous phase") and a cholate-rich phase (designated as the "cholate phase"), which during gel-formation gradually increases in bulk until its parts are more or less coterminous throughout the system. When this gel is introduced into a

* 'Roy. Soc. Proc.,' B, vol. 87, p. 366 (1914).

salt solution a third phase is introduced, so that the total effect of erosive salt action must be studied in a triphasic system.

The cholate phase of the gel consists apparently of heavily hydrated aggregates of cholate salt which may vary (*a*) in composition (nature of cholate salt, degree of hydration, etc.), (*b*) in state of aggregation. It is assumed that it is upon these two factors the stability of the gel structure will mainly depend, and it remains to be considered how they may be affected by the salts in the triphasic system in which the erosion is studied. The possible actions of the salts are considered under six headings.

I. *The Influence of the Salts on the Cholate Component in the Cholate Phase.* —In the ordinary method of the formation of the gel, there is a reaction between calcium chloride and sodium cholate. The nature of this reaction is not, so far, clear. It is not an ordinary double decomposition, with the formation of calcium cholate as an insoluble gel-like substance. Such a method of gel-formation has been discovered in another system under investigation by one of the authors of this paper (S. B. S.), who has found that when calcium chloride is added to a solution of the sodium salt of plant pectin (which is an acid) a gel is produced immediately by precipitation. The cholate gel, on the other hand, forms only slowly at ordinary temperatures. It is not possible to state at the present what proportions of the cholate are present in the cholate phase as calcium and sodium salts. Probably the reaction between calcium chloride and sodium cholate may be represented in the form of a reversible reaction.

$$2C_{24}H_{39}O_5Na + CaCl_2 \rightleftarrows (C_{24}H_{39}O_5)_2Ca + 2NaCl.$$

The addition of sodium chloride in excess, or of other chlorides, would, if this equation truly represents the facts, influence the composition of the cholates formed. Further work is necessary to determine the composition of the phases produced in the process of gel-formation.

II. *Influence of Salts on the Composition of the Two Phases of the Gel Resulting from the Establishment of Osmotic Equilibrium between these Phases.*—The cholate gel is an example of the special case in which the action of a non-diffusible ion (cholate) holds electrostatically a diffusible one (the metallic ion Ca, K, Na, etc.). The distribution of the components between the two gel phases to establish osmotic equilibrium would probably proceed in accordance with the hypothesis of Donnan, elaborated in the course of his investigations of membrane equilibria and potentials in the presence of non-dialysable electrolytes.*

III. *Influence of the Salts on the Electric Charge on the Particles of the*

* Donnan, 'Zeitsch. f. Elektrochemie,' vol. 17, p. 572 (1911).

Cholate Phase of the Gel.—It is a well-established fact that the state of aggregation of a colloid is subject to the influence of the charge it carries, and that this charge can be affected by the presence of salt ions. The action of these is a function of their nature and valency, the multivalent ions exerting the greatest amount of influence. The suspensoid (lyophobe) colloids are more especially sensitive to this particular action of salt ions, but it has been shown by Mines* that the hydrated (emulsoid or lyophil) colloids are by no means insensitive. Preliminary experiments indicate that the cholate gel is affected by the valency of the anions, but experiments dealing with this possible factor are not yet complete.

IV. *The Influence of Salts on the Distribution of Water between the Two Phases of the Gel.*—As an extreme instance of the action of salts on the distribution of water between two phases, the salt precipitation of proteins may be quoted. This action is ascribed to the withdrawal of water from the protein phase† and the efficiency of salts in producing such an action is, most probably, a function of their state of hydration in solution. It is conceivable that in a two-phased gel system salts may exert an analogous action (by withdrawing water from the phase containing the larger amount of the substance to which gel formation is due, in the special case under consideration the cholate phase), although the effects may not be so readily demonstrable as in the case of proteins and only be revealed by a detailed analysis of the separate phases.

V. *Influence of Salts due to their Effect on the Surface Tension of Water.*—It has been shown that the greater the lowering of the surface tension of water produced by dissolving a given salt, the greater is the disaggregating action of the solution.‡ It has already been pointed out that the rate of formation of the cholate gel by different calcium salts is partly a function of the surface tensions of their solutions; the lower the surface tension of the calcium salt solution, the more slowly does gel-formation take place. In the case of certain calcium salts, gel-formation can be inhibited entirely when the concentration of the calcium salts is sufficiently great.§ Some experiments on the reversal of the gel by salts, which are not given in detail in this paper, indicate that, at any rate in the second phase of the erosion curve, the action of the salts which markedly lower the surface tension of water is on the whole greater than those which produce aqueous solutions of higher

* 'Kolloidchemische Beihefte,' vol. 3, p. 191 (1911–12).

† See especially Harriette Chick and C. J. Martin, 'Biochem. Jour.,' vol. 7, p. 380 (1913).

‡ For examples see Schryver, 'Roy. Soc. Proc.,' B, vol. 83, p. 96 (1910).

§ Schryver, 'Roy. Soc. Proc.,' B, vol. 87, p. 366 (1914).

surface tension. The viscosity of salt solutions also probably plays some part in that disaggregating action of salts which is connected with their effect on surface tension of water, owing to their forming a kind of diffusion layer round the colloid phase. This action has been already discussed in some detail in an earlier paper.*

VI. *Influence of Salts due to Differences in Diffusion Rates.*—Salts will diffuse from one phase to another at different speeds, and the rate of interchange between the phases will be a function of this factor, which is such an obvious one that it needs no detailed discussion. It is possible that salts also exert other actions which have not been enumerated here.

From the foregoing remarks it would appear probable that the action of salts in a gel system is very complex and that it is hardly possible to investigate each single factor in a system such as that of the cholate gel; the results recorded in this paper must, therefore, be regarded as somewhat empirical in character. In this and in the earlier papers of this series on gel-formation, attention has been repeatedly drawn to the analogies between the physical characters of the cholate gel and certain vital activities of the cell, and if, as has been suggested, the protoplasm and the cell membrane contain a gel structure like that of the cholates, it is obvious that the biological action of salts must be a very complex one, which cannot be ascribed to the action of any one single factor acting separately. It is hoped that by a continuation of this work more light may be thrown on certain important physiological problems, especially those connected with the permeabilities of cells and their functional changes. Attempts have already been made to trace further analogies between the action of salts in producing such changes and their erosive action on cholate gels.

SUMMARY.

1. The erosive action of chloride solutions on a cholate gel containing added chlorides has been investigated. If the concentrations of the eroding solutions are plotted as abscissæ and the amounts of erosion as ordinates, a curve is obtained which is of diphasic character. The amount of erosion increases with increasing concentration to a maximum, and then, with further increase in the concentration of the eroding solution, it diminishes to a minimum; on increasing the concentration beyond this latter point, the amount of erosion increases continuously. The portion of the curve between the two minimal points is designated the "zone of instability" of the gel.

2. The breadth of this zone and the amount of erosion within it is a

* Schryver, 'Roy. Soc. Proc.,' B, vol. 83, p. 96 (1910). See also Traube, 'Internationelle Zeitsch. f. physikalisch-chemische Biologie,' vol. 1, p. 275 (1914).

function of the amount of salt added to the gel; the larger this amount, the broader the "zone of instability" and the greater the amount of erosion within it.

3. An erosion curve of similar form is obtained when a non-electrolyte (dextrose) is added to the gel. The sugar is, however, without action when present in the eroding fluid, whether the gel contains added salts or not.

4. Quantitatively, the chloride solutions in their erosive action differ considerably from one another; the differences between their action is less marked when present in the gel. The maximal effect, both as regards the breadth of the "zone of instability" and the amount of erosion within this zone, is produced by lithium chloride. The order of action of the salts investigated is $LiCl > NaCl$ (generally) $> MgCl_2 > KCl$. This is the order of their action in increasing the permeability of certain vegetable cells.

5. Relatively more calcium chloride is necessary to "antagonise" the erosive action of salts within the "zone of instability" than is necessary to antagonise the same amount of erosive action produced by higher concentrations (in the second phase of the erosion curve).

6. The erosive action of sodium salts other than chloride on the gel containing added chlorides was also investigated, and an erosion curve of diphasic character was always obtained. The form differed for each salt, both as regards the breadth of the "zone of instability" and the amount of erosion. Specially remarkable was the erosion curve produced by sodium lactate on a gel containing potassium chloride. The erosion curve in this case showed a very narrow "zone of instability," with an exceptionally large amount of erosion within the zone.

7. The general action of salts on a gel system has been discussed, and it is pointed out that it is probably the resultant of several physico-chemical factors acting simultaneously. Attention is also called to the probable complexity of the biological action of salts.

Selective Permeability: The Absorption of Phenol and other Solutions by the Seeds of Hordeum vulgare.

By Adrian J. Brown, F.R.S., and Frank Tinker, M.Sc.

(Received July 24, 1916.)

Previous researches on the absorption of various aqueous solutions by the seeds of Hordeum (barley) have been directed mainly to a study of the phenomena attending the diffusion of the solvent through the semipermeable covering of the seeds.* The latter, however, is permeable not only to the water but also to certain classes of solutes in solution, such as the phenols and fatty acids; in fact, the membrane behaves in their presence as a selectively permeable one rather than a semipermeable one. In the present communication we deal more particularly with the extent to which certain of these permeable solutes enter the seeds together with the water, in the hope that the results may throw some light on the physical causes governing the phenomenon of selective permeability.

A preliminary investigation having given the somewhat striking result that phenol and aniline solutions enter the seeds in higher concentration than the solutions in which they are immersed, we decided to make a series of quantitative experiments with solutions of aniline, phenol and acetic acid of various concentrations.

1. *Extent to which Aniline, Phenol and Acetic Acid enter the Seeds from Solutions of Various Strengths.*

(a) *Aniline Solutions.*—The seeds were steeped in approximately N/4, N/8, N/12, and N/16 solutions respectively for two days.

The amount of aniline which had entered was found by hydrolysing the seeds with dilute sulphuric acid. This operation, which was performed under reflux at about 80° C., was complete in from two to three days' time. After filtering, the solution was made up to a known volume, and the aniline precipitated and weighed as tribromaniline.† The accuracy of the method was tested by determinations of aniline solutions of known strengths, both alone and in presence of barley seeds which had been hydrolysed to sugar. The presence of the latter was found to have no influence on the results, which were found to be accurate to 3 or 4 per cent.

The results are given in Table I.

* A. J. Brown, 'Roy. Soc. Proc.,' B, vol. 81, p. 82 (1909); *ibid.*, with F. P. Worley, B, vol. 85, p. 546 (1912); *ibid.*, with F. Tinker, B, vol. 89, p. 119 (1915).

† *Cf.* section on Aniline in Allen's 'Commercial Organic Analysis.'

Table I.—Seeds in Aniline Solutions of Various Strengths.
Temperature 15° C.

Strength of aniline solution outside seeds.	Gain in weight of 5 grm. seeds after 2 days.	Weight of aniline found inside seeds.	Weight of water inside seeds.	Strength of solution inside seeds.	Ratio of strength solution inside to strength solution outside.
	grm.	grm.	grm.		
N/4 (2·30 grm. per 100 grm. water)	2·724	0·184	2·540	7·25 grm. per 100 grm. water	3·15
N/8 (1·16 grm. per 100 grm. water)	2·564	0·087	2·447	3·52 grm. per 100 grm. water	3·04
N/12 (0·87 grm. per 100 grm. water)	2·909	0·067	2·842	2·35 grm. per 100 grm. water	2·70
N/16 (0·575 grm. per 100 grm. water)	2·851	0·042	2·840	1·47 grm. per 100 grm. water	2·55

In each instance the aniline solution is stronger within the seed than in the outside solution. Moreover, the aniline solution inside the seeds becomes stronger as the outside solution becomes stronger, and is in each of the experiments about three times as strong as the latter. Further, it will be noticed that the solution diffusing into the seeds from N/4 aniline solution contains more aniline than does a saturated solution, *i.e.*, the adsorbed solution is highly supersaturated.*

(b) *Phenol Solutions.*—The procedure was exactly the same as in the case of aniline, the phenol being estimated as tribromphenol.† The results are given in Table II.

Manifestly concentration of phenol within the seeds takes place as in the case of aniline. The high degree of supersaturation of the solution adsorbed from the N/2 phenol is also very marked.

(c) *Acetic Acid Solutions.*—Acetic acid, unlike aniline and phenol, is miscible with water in all proportions, so that solutions of any chosen concentrations from 0 to 100 per cent. acetic acid can be employed.

To determine the concentration of the adsorbed solution within the seeds, the latter were hydrolysed with a known volume of standard sulphuric acid, the solution filtered, made up to a known volume (250 c.c.), and titrated with standard NaOH, correction being made for the sulphuric acid employed

* This fact possibly indicates that the solubility of the aniline is increased in the adsorbed film in contact with colloidal surfaces.

† A small correction had to be made for the solubility of the tribromphenol in water. When this was done the accuracy of the results was about the same as for aniline (3 or 4 per cent.).

Table II.—Seeds in Phenol Solutions of Various Strengths.

Strength of phenol outside seeds.	Gain in weight of 5 grm. seeds after 2 days.	Weight of phenol inside seeds.	Weight of water inside seeds.	Strength of solution inside seeds.	Ratio of strength solution inside to strength solution outside.
	grm.	grm.	grm.		
N/2 (5·00 grm. per 100 grm. water)	3·321	0·485	2·836	17·2 grm. per 100 grm. water	3·4
N/4 (2·50 grm. per 100 grm. water)	3·081	0·243	2·838	8·6 grm. per 100 grm. water	3·4
N/8 (1·24 grm. per 100 grm. water)	3·024	0·129	2·895	4·45 grm. per 100 grm. water	3·5
N/16 (0·64 grm. per 100 grm. water)	3·205	0·057	3·148	1·81 grm. per 100 grm. water	2·9

for the hydrolysis. The accuracy of the method was tested in the same way as in the case of aniline and phenol, and the error found to be within 5 per cent.

Table III.—Seeds in Acetic Acid Solutions of Various Concentrations.

Strength of outside solution x per cent. acetic acid to $(100-x)$ per cent. water.	Gain in weight of 5 grm. seeds after 2 days.	Strength of solution inside seeds.	Ratio of strength solution inside to strength solution outside.
	grm.	per cent.	
0	2·640	—	—
10	2·643	8·1	0·81
20	2·816	27·0	1·35
30	3·028	38·3	1·27
40	3·037	53·4	1·33
50	2·974	80·1	1·60
60	3·052	80·0	1·33
70	2·862	81·8	1·17
80	2·590	82·5	1·03
90	2·164	80·0	0·89
100	0·040	—	—

The graphical relation between the strength of the solution inside the seeds and that outside is shown in Diagram I. It will be seen that for all outside solutions stronger than 50 per cent., the strength of the adsorbed solution inside the seeds is constant at 80 per cent. Apparently the colloidal contents of the seeds cannot take up acetic acid unless at least 20 per cent. of water diffuses with the acid also. A striking confirmation of this is found in the absorption curve for water-acetic acid mixtures. Table IV

shows some typical absorption results, whilst Diagram II shows them graphically. The falling off of the absorption rate and also of the weight

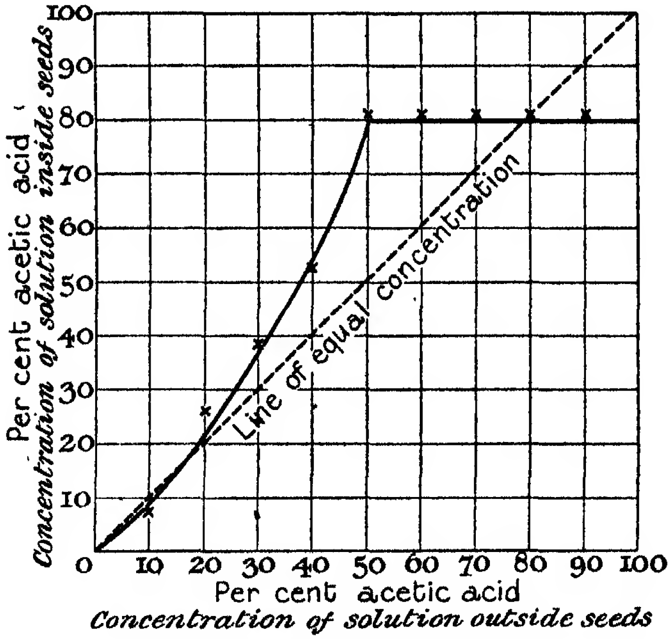

Diagram I.—Acetic acid solutions.

of solution absorbed is very noticeable above the 80 per cent. mark. Pure acetic acid is not absorbed by dry seeds at all.*

Table IV.—Absorption Curves for Water-Acetic Acid Mixtures.

Strength of acetic acid solution.	Percentage gain in weight of seeds, due to absorption of solution after				
	2 days.	3 days.	6 days.	9 days.	13 days.
per cent.					
0	36·4	48·6	55·8	64·5	68·3
20	37·0	49·6	59·8	66·7	69·4
38·5	33·1	45·2	52·3	63·0	68·9
55·5	31·7	44·6	52·4	63·8	70·6
71·4	32·9	45·3	58·7	66·5	74·3
83·3	27·8	43·2	49·7	61·9	68·3
90·9	13·5	19·8	26·8	43·4	55·0
100	5·5	9·2	8·9	10·9	13·5

* *Loc. cit.*, A. J. Brown, 'Roy. Soc. Proc.,' B, vol. 81, p. 82 (1909).

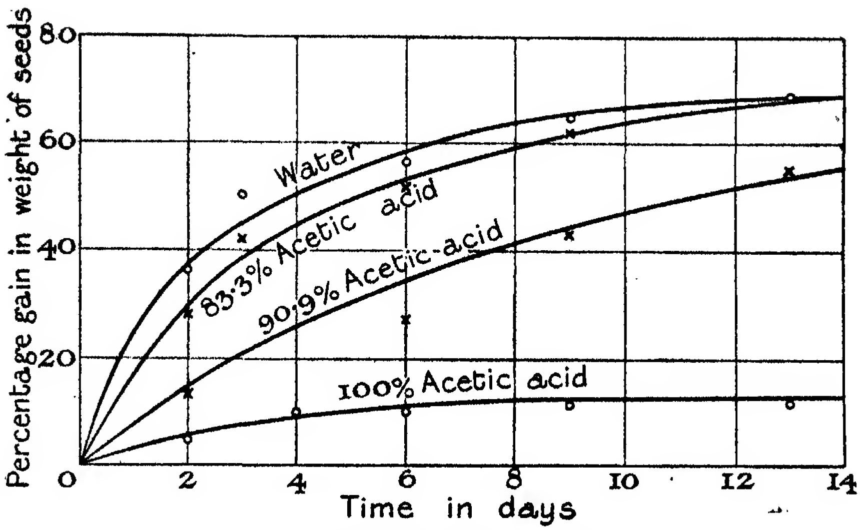

DIAGRAM II.—Absorption curves for water–acetic acid mixture.

2. *Relation between the Concentration of the Various Inside Solutions and the Amount Absorbed at Equilibrium.*

A comparison of these two quantities for acetic acid (Tables III and IV) shows that when the solution inside is stronger than that outside, the amount asborbed at equilibrium is greater than the amount of pure water absorbed when the seeds are in water only. Furthermore, as the relative concentration of the inside solution increases, the equilibrium point rises also, and is at a maximum when the relative concentration of the inside solution is at a maximum (with outside solutions of from 50 to 70 per cent. strength). In the same way, the equilibrium point is the same as that for pure water when the outside and inside solutions have the same concentration (80 per cent.). When the solution inside is weaker than that outside (at from 80 to 100 per cent. outside concentration), the equilibrium point is lower than it is for pure water.

Similar considerations hold for the solutions of aniline and phenol. They enter the seeds in a concentration greater than that of the outside solution, and the equilibrium point is higher than for pure water in their case also.*

Evidently the amount of solution taken up by the seeds at equilibrium is determined by the difference between the inside and outside concentrations. The equilibrium point is lower, equal to, or higher than it is for pure water according as the inside solution is weaker, the same strength as, or stronger than the outside solution.

* A. J. Brown and F. Tinker, 'Roy. Soc. Proc.,' B, vol. 89, p. 119 (1915).

Conversely, the position of the equilibrium point gives an indication of the extent to which the solute enters the seeds. Table V shows a series of organic solutes arranged approximately in the order of the equilibrium points they give,* and therefore in the order of the extent to which the solute enters. The surface tensions of the solutions are given in certain cases also.†

Table V.—Showing Order of Extent to which Various Organic Solutes Enter the Barley Seeds.

Solute (molar).	Percentage increase in weight when seeds are immersed in molar solution.	Surface tension of molar solution (Traube).
		dynes/cm.
Cane sugar	39·8	76·5
Dextrose	40·8	75·7
Glycerol	41·5	74·1
Glycine	41·8	75·8
Tartaric acid	42·2	—
Urea	45·2	74·8
Ethylene glycol	52·7	71·8
Lactic acid	61·4	—
Glycollic acid	63·4	—
Acetaldehyde	66·6	—
Acetone	68·7	56·2
Acetic acid	68·0	61·5
Ethyl alcohol	69·6	57·9
Ethyl acetate	71·8	43·2
N/2 phenol	85·0	48·3

The explanation of this relation between the relative concentrations of the inside and outside solutions is quite simple. When there is a difference between the two concentrations in question, an extra osmotic force is introduced into the system beyond the forces which are present when there is no concentration difference. If the seeds are immersed in pure water, for instance, diffusion merely takes place until the surfaces of the colloidal contents are saturated with moisture. But, if the seeds are immersed in a solution, and the membrane acts selectively towards the latter, there is an outward or inward osmotic force according as the solution inside is weaker or stronger than that outside. In the former case the colloidal contents of the seeds will be prevented from taking up as much liquid on to their surfaces as they would otherwise do, so that the equilibrium point will be lowered. In the latter case still more solution will be driven into the seeds, and the equilibrium point raised.

* This Table is compiled from a previous paper, 'Roy. Soc. Proc.,' B, vol. 81, p. 82 (1909). The order is only approximate.

† The surface tensions are taken from J. Traube's paper, 'Journ. Phys. Chem.,' vol. 14, p. 460 (1910), and other papers.

3. *Relation between the Selective Adsorption of the Various Solutions and their Surface Tensions.*

An inspection of Table V shows that the solutes which are most strongly adsorbed by the seeds of Hordeum, such as aniline, phenol, and the organic acids, are those which give solutions having very low surface tensions. On the other hand, those solutes which are not adsorbed, such as the sugars and the polyhydric alcohols, give solutions which have relatively high surface tensions. In fact, the order giving the extent to which the solutes are adsorbed is almost the same as that for the surface tensions. Evidently, Gibbs' well-known rule connecting adsorption and surface tension is applicable with a fair degree of accuracy to adsorption by the colloidal contents of barley seeds.*

Most important of all, the fact that the solutes giving high surface tensions do not, as a rule, penetrate the membrane, suggests that the selective action of the latter is due to selective adsorption. This hypothesis accounting for the selective properties of membranes of the copper ferrocyanide type has already been advanced by one of us on other grounds.† The barley membrane, like almost all other membranes, is colloidal in nature, *i.e.* it is composed of aggregations of colloidal particles enclosing minute capillaries. Hence any liquid which enters its capillaries is adsorbed liquid; and that selective adsorption of solvent or solute into the capillaries will take place is practically certain.‡ The results of the present investigation indicate that these selective adsorption effects are both varied and considerable in magnitude; and great enough to account for the selective action. The kind and degree of the selective condensation at the surface of the membrane may not be exactly the same as at the surface of the starch granules, and both may differ from the selective effects at a solution-air interface. But the general agreement of each with Gibbs' relationship shows that a great similarity exists between them.

* The investigators have found this rule to be more or less applicable to many other boundary surfaces. *Vide* W. C. McLewis "On the Adsorption of Various Solutes at a Hydrocarbon Oil-Water Interface," 'Phil. Mag.' (6), vol. 15, p. 449 (1908); *ibid.* (6), vol. 17, p. 466 (1908).

† Tinker, 'Roy. Soc. Proc.,' A, vol. 92, p. 357 (1916).

‡ *Loc. cit.*, the paper just mentioned.

Methods of Raising a Low Arterial Pressure.

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CONTENTS.

In the course both of physiological experiments and of clinical practice a low arterial pressure may be due to two different causes, apart from failure of the heart.

After hæmorrhage, the blood pressure is low on account of deficiency of blood in circulation, so that the output of the heart is decreased and is insufficient to keep up a due supply in the arteries to take the place of that flowing through the capillaries. Now, while it is obvious that constriction of the arterioles would raise the pressure in such cases, by diminishing the rate of outflow through the capillaries, the result would be to decrease the supply of blood to all organs whose arterioles are affected, so that no real gain would be obtained. In such cases, what is needed is to increase the volume of blood, without constricting arterioles.

But the arterial pressure may be low, although the volume of the blood is normal. Supposing that the heart is beating efficiently, the low pressure is, in such cases, due to peripheral vaso-dilation. Such a condition is met with when the spinal cord is cut high up, as in the "spinal animal," and also in cases which are called "shock." Here, what is required is clearly to restore the normal tonic contraction of the arterioles. An increased volume of blood may be useless or even harmful, if the heart cannot correspondingly increase its output.

It is, nevertheless, the usual practice to treat both kinds of state by the intravenous injection of saline solutions, sometimes by transfusion of blood. But the unsatisfactory effect of saline injections suggested that a more efficient fluid might be found and the following investigation was undertaken with that object.

Loss of Blood.

I will consider first the restoration of arterial pressure after loss of blood.

Experiments were made to begin with in order to analyse the effects produced by injection of pure saline solutions.

A cat was anæsthetised by chloroform and ether and afterwards by urethane intravenously (1 grm. per kilogramme body weight), given slowly. Cannulæ were placed in the carotid artery for the registration of the arterial pressure, in the external jugular vein for the introduction of liquids from a burette, and in the femoral artery for the withdrawal of blood. The vagus nerves were cut in order to avoid cardiac and depressor reflexes. The blood pressure at the beginning of the experiment was 158 mm. of mercury. This was reduced to 46 mm. by the removal of 66 c.c. of blood. The same volume of Ringer's solution, warmed to 38° C., was then run into the vein. The blood pressure returned for a brief period to 128 mm.; that is, there was a restoration of three-quarters of the amount by which it had fallen. The size of the heart beats, which had been greatly reduced by the loss of blood, became as large as at first. But this partial recovery was only maintained for about five minutes and by the end of 24 minutes the pressure had fallen again to 80 mm., being only half of the original height.

In other experiments of the same kind, it was found that the fall of blood pressure resulting from hæmorrhage was restored by about two-thirds to three-quarters of the amount by which it had fallen. Of course, by the injection of amounts of saline solution greater than the volume of the blood removed, a temporary rise to the normal value may be obtained. But it is undesirable to augment the total volume of blood on account of the strain on the heart, and it is important to know why the restoration of the blood to its normal quantity, if done by saline injection, does not result in a return to the corresponding initial pressure.

Downs (12) found that, if the arterial pressure had fallen to three-eighths of the normal by hæmorrhage, injection of saline could only raise it to about two-thirds again. He gives detailed measurements of the relative effects obtained in various degrees of loss of blood.

As will be seen presently, there are two separate phenomena requiring explanation. Why is saline solution relatively ineffective in restoring pressure? And, secondly, why does the pressure actually produced fall again more or less rapidly to a value very little higher than that before the injection.

In regard to the former problem, we know that, the cardiac output being supposed constant, the height of the arterial pressure depends on the

resistance in the peripheral arterioles and that this resistance is entirely due to the internal friction or viscosity of the blood. The rate of flow is inversely proportional to the viscosity and directly proportional to the driving pressure, by Poiseuille's law. This law has been shown by Du Bois-Reymond, Brodie, and Müller (1) to apply to the circulation, contrary to the objections of Heubner and others. The output of the heart being constant, by hypothesis, the rate of flow through the blood-vessels must also be constant, and therefore if the viscosity of the blood decreases, the driving pressure must decrease also. Otherwise, the current through the capillaries would be larger than that supplied by the heart. In other words, the arterial pressure must fall. Although this fact seems obvious, I thought it of interest to test it in a model made of indiarubber tubing connected with a glass tube of 0·97 mm. bore and 27 cm. long. Through this a current of gum solutions of various concentrations and viscosities was driven by means of a small pump of constant delivery of 0·3 c.c. per stroke. The outflow from the capillary was found to be constant, although the driving pressures ranged from 178 mm. of mercury, when the viscosity was 3·7 times that of water, down to 45 mm. of mercury with water itself.

Now, the viscosity of blood is considerably higher than that of water or of a dilute saline solution. In the cats used in my experiments it was usually about three times that of water, but in one case it was only 2·2 times, and in another it was as high as four times. The viscosity of Ringer's solution, on the other hand, is practically the same as that of water. It follows that, if part of the blood is replaced by such a saline solution, the resulting viscosity is correspondingly reduced, and, other things equal, the arterial pressure must decrease. Thus, in one experiment, the viscosity was reduced to 1·6 times that of water by replacing half of the blood by Ringer's solution. In another case it was reduced from 2·2 times to 1·8 times by the replacement of 47 per cent. of the calculated blood volume by the saline solution.

The viscosities were determined at 38° in an apparatus similar to that described by Scarpa (2), in which a known volume is driven first upwards through a capillary tube by pressure and then allowed to run back downwards. The mean of several pairs of readings was taken. The advantage of this method is that no correction need be made for specific gravity or surface tension, since these factors correct themselves by acting in opposite directions in upward and downward movement.

The blood volume was taken to be 48 c.c. per kilogramme of body weight.

In one experiment several samples of blood of 45 c.c. each were removed and replaced by saline in series, so that the viscosity decreased each time. The height of the arterial pressure, compared with the viscosity in each stage, was as follows:—

Table I.

Blood pressure.	Viscosity relative to water.	Relative blood pressure.	Relative viscosity.
136	3·0	100	100
128	2·65	94	89
104	2·3	77	77
82	2·0	60	66

It is evident that the diminution of viscosity is a sufficient explanation of the inefficiency of saline solutions, so far as the immediate effect is concerned. If, therefore, the viscosity of Ringer's solution could be raised by the addition of some innocuous substance, a much better result would be obtained. There are several substances which might be used for this purpose.

Soluble starch has the disadvantage of not having sufficient viscosity, except in concentrated solutions. It does not give very homogeneous solutions, and alters by keeping, even in the cold. The solution becomes acid, as shown by Wolff and Fernbach (3), by separation of phosphoric acid, so that neutralisation is necessary each time that it is used. It has, further, the objection of a very small osmotic pressure. The importance of this fact will be seen presently.

Amylopectin, prepared by precipitation of ordinary starch with acetone, in the manner of Wolff and Fernbach, has a high viscosity, but an insignificant osmotic pressure.

Agar requires too high a temperature to melt the jelly, and is practically devoid of osmotic pressure.

Gum acacia in 7-per-cent. solution has a viscosity about equal to that of blood. The commercial samples consist partly of the calcium salt. I find that the one used in my experiments had a calcium content equal to 2·23 per cent. of calcium chloride. A 7-per-cent. solution would therefore contain 0·16 per cent. This is too high for a normal physiological solution. It is advisable to precipitate by the addition of the necessary amount of phosphoric acid; 7 grm. of the gum referred to require 9·38 c.c. of decimolar phosphoric acid. On making just alkaline to neutral red with sodium hydroxide, the calcium phosphate is precipitated and falls on standing. The solution consists now of the sodium salt of the gum acid, saturated with calcium phosphate. A saturated solution of calcium phosphate was found by Ringer to contain the optimal concentration of calcium. The sodium salt has the further advantage of a slightly higher viscosity than the calcium salt. To complete the solution, the correct amount of potassium

chloride is finally added. For clinical use, sterilisation by heat does not perceptibly diminish the viscosity. The mucilage of the British Pharmacopœia, when diluted by adding 400 c.c. of water to 100 c.c., makes a 7-per-cent. solution.

Finally, *gelatin* is very convenient in many ways, as solutions are quickly made. If the maximum viscosity is required, a temperature above 40° C. should not be employed in making the solution. If heated to 100°, as Moore and Roaf (4) showed, the osmotic pressure rises. The decrease of viscosity on heating is a disadvantage for clinical use, since sterilisation is indispensable, owing to the usual presence of micro-organisms, especially those of tetanus.

The Table below gives numerical data of the properties of various solutions, so far as they concern us here.

Table II.

	Viscosity ($H_2O = 1$).	Viscosity in dynes × 10^3 ($H_2O = 6 \cdot 6$).	Osmotic pressure against water.	Osmotic pressure against Ringer solution.
			mm. Hg.	mm. Hg.
Blood of cat	3·0	19·8	—	—
Ox serum	1·5	9·9	116	36–40
Soluble starch (Kahlbaum) 4 per cent.	1·7	11·2	—	14–16
Soluble starch (Kahlbaum) 4 per cent., after cold storage	2·26	16	—	—
Wheat starch, 2 per cent.	2·65	17·5	—	—
Amylopectin, 1·72 per cent.	4·8	32	—	—
Gum acacia, 7 per cent., Ca salt	3·1	20·5	—	—
Gum acacia, 7 per cent., Na salt	3·4	22·5	218	89
Gelatin, 6 per cent.	4	26·4	95	38·5
Gelatin, 6 per cent., after heating to 100°	3	19·8	—	63

Although some of these figures are to be found in the literature, I thought it better to determine them all with the solutions actually used in my experiments, and under similar conditions at 38°. Many of the data are new. Others differ somewhat from previous determinations, as would be expected from the variability of colloidal substances.

In experimental test, it was found that injection of Ringer's solution containing one or more of these substances in sufficient quantity to raise the viscosity to that of blood, even when injected only in amount equal to that of the blood lost, brought back the arterial pressure to its original height,

and sometimes temporarily above this height. The explanation of this latter fact will be seen immediately. Thus:—

A cat with a blood pressure of 110 mm. of mercury had about half of the calculated amount of its blood removed. The pressure fell to 40 mm. It was brought back at once by the injection of an equal volume of Ringer's solution containing gum in sufficient quantity to raise its viscosity to 3·1 times that of water. In the course of two or three minutes the pressure rose further to 145 mm. Although it soon commenced to fall slowly, it was still at its initial value of 110 mm. at the end of 43 minutes. It had only fallen to 102 mm. in an hour and to 98 mm. in 75 minutes. Similar results were obtained with gelatin.

We see that by raising the viscosity of the injected fluid to that of blood by the addition of gum or gelatin, the blood lost can be replaced by an equal volume of the solution, with a return to its original height. Further, that this height is maintained for an hour or so, and even then has only fallen to an unimportant degree.

Pure Ringer's solution, as shown above, is very inefficient in maintaining the blood pressure even at that height to which it at first raises it. Why do gelatin and gum behave differently in this respect? It is clear that viscosity alone is not a sufficient explanation. The fact that gelatin solutions caused a more permanent rise of blood pressure than pure saline was noted by J. Hogan and Martin H. Fischer (9), and Bogert, Underhill, and Mendel (17) found that saline solutions containing 2 per cent. of gelatin did not leave the blood-vessels as rapidly as pure saline.

A partial explanation of these facts is given by Knowlton's experiments on the secretion of urine (5). Starling (6) had shown that if the osmotic pressure of the blood colloids, to which the membrane of the glomerulus is impermeable, be reduced by dilution of the blood, diuresis results. This is the case when a pure saline solution is injected. But Knowlton showed that by the addition of a colloid which has an osmotic pressure, such as gelatin, the effect of the dilution is greatly decreased. Further, if a colloid which has no perceptible osmotic pressure, such as the soluble starch used by him, be added, instead of gelatin, the diuresis is as great as with saline. This is confirmed by the following experiment:—

A cat with a blood pressure of 180 mm. of mercury had one-third of its blood removed. An equal volume of 5-per-cent. soluble starch in Ringer's solution was injected when the blood pressure was 70 mm. It was brought back, temporarily, to 160 mm., the viscosity of the solution being only 2·2 times that of water. The blood pressure then began to fall, becoming 130 mm. in 18 minutes, and 62 mm. in 40 minutes. At the end of the

experiment, 50 c.c. of urine were found in the bladder, while 30 c.c. had been passed in the course of the experiment.

Other experiments were made with the insertion of a cannula in the bladder, and records made by means of an electrical drop recorder. It was found that while saline solutions produced diuresis, this was not the case with gelatin. For example, only 14 c.c. were excreted in 68 minutes, against 2·5 c.c. in six minutes (=14 c.c. in 34 minutes) before the bleeding.

The loss of the injected fluid by renal excretion does not, however, explain the phenomenon of the fall of blood pressure. It was sometimes found in my experiments that very little urine was produced after the saline injection. This was probably due to the kidney having suffered from want of oxygen during the period of low blood pressure following the removal of blood. In fact, it was noticed in one case that a slow renal secretion subsequent to a saline injection was considerably increased by a more vigorous artificial respiration, although there had been no signs of asphyxial stimulation of the nerve centres. It is well known that the kidney is sensitive to deficient supply of oxygen. But what concerns us for the present purpose is the fact that, although there may be no increased loss of fluid by renal excretion, yet the arterial pressure falls rapidly after saline injections.

The additional factor is, no doubt, passage of fluid into the tissues. Bogert, Underhill and Mendel (17) have shown that saline solutions pass into the tissues rapidly and that the kidney is not necessary for the removal of excess of fluid from the circulation after intravenous injections into the normal animal. Moreover, the production of œdema by perfusion with Ringer's solution is familiar to all who have made such experiments. The liver, in particular, swells to a great extent when saline solutions are injected. This is obvious to the eye, and in some plethysmographic experiments on a lobe of the liver which I made some years ago, I noticed a very considerable increase in volume under such circumstances. In the experiments to be referred to below, in which a limb was perfused with Ringer's solution containing 7 per cent. of gum acacia, the absence of œdema was noticeable. We have also seen above that the blood pressure remains high for a long time after the injection of gum or gelatin, but falls after starch solutions. In what way, then, do gum and gelatin differ from starch? Clearly in the possession of osmotic pressure. Starling (7) has emphasized the importance of the osmotic pressures of the protein content of blood and tissue fluids in the passage of water from one to the other. The protein content of the blood plasma is higher than that of the tissue lymph, so that there is a continual attraction of water from the tissues to the blood. This is, however, normally balanced by filtration in the other direction, which occurs where the pressure in the

blood-vessels exceeds the difference between the osmotic pressure of their contents and that of the tissue fluids. If, on the other hand, the blood is diluted, so that the osmotic pressure of its colloids is lowered, an internal pressure of the same height as before will cause greater filtration, and, at the same time, the difference between the osmotic pressure of the blood and that of the tissue fluid being less, there is a decreased osmotic attraction of water by the blood from the tissues. The two causes combine to produce œdema. The colloid added to increase the viscosity of an intravenous injection must therefore possess an osmotic pressure equal to that of the colloids of the blood. Table II, above, includes some determinations of the osmotic pressures of certain solutions of interest in the question. These were all made under the same conditions with Moore and Roaf's osmometer, arranged for changing the outer fluid as required, practically as described by me in a previous paper (8). It will be noticed that the value found for serum against Ringer's solution is the same as that found by Starling, but that against distilled water the reading is considerably higher. The water and the saline solution used were made just faintly alkaline to neutral red in order to approximate to the reaction of the blood, and also to avoid the loss of cations from the colloidal salt which occurs when the outer solution becomes acid from any cause, such as absorption of carbon dioxide from the air (see my paper [10, p. 251]). The fact that the osmotic pressure of gelatin is lowered by salts was noticed by Moore and Roaf, and interpreted as due to the aggregation of the colloid. I accepted this explanation at first, but subsequently found that a sufficient explanation is to be found in the unequal distribution of the salts between the two sides of the membrane, when the colloid is itself a salt of a colloidal acid with a diffusible cation. The explanation is discussed in my 'General Physiology' (pp. 120, 160, 161). Owing to the small molecular weight of the salts in Ringer's solution, a very small difference of concentration in favour of the outer fluid suffices to produce a considerable fall in the apparent osmotic pressure of the colloid. In the case of the sodium salt of gum, for example, there is a difference of 180 mm. of mercury between the osmotic pressures against Ringer's solution and against water. Supposing the sodium chloride in the former case were 90 per cent. dissociated, a difference in concentration between the inner and outer liquids of 0·034 per cent. in sodium chloride, or 3·8 per cent. of the total concentration, would account for the difference in osmotic pressure. Thus, the osmotic pressure of the contents of the osmometer is the sum of those of the colloid and the salts; the osmotic pressure of the outer fluid is that of a solution of the salts of a slightly higher concentration; the pressure shown by the manometer is the difference between the two. It is interesting

to note that the difference referred to is greater in the case of gum than in the case of serum proteins or in that of gelatin. I take it that this is due to the acid of the gum being a stronger one than those of the proteins, so that its salts are more dissociated electrolytically. The difference in the case of congo-red is still greater.

The question is, then, Are we to take as the required colloidal osmotic pressure of our ideal injection fluid, that against water or against Ringer's solution? If the wall of the blood-vessels consists of a membrane permeable to crystalloids, impermeable or nearly so to colloids, it will behave as the parchment paper membrane of our osmometer, and we must take the osmotic pressures as measured against Ringer's solution as those which come into play. We require a solution of a colloid which gives under these conditions an osmotic pressure of about 40 mm. of mercury. This, as Table II shows, is given by a 7-per-cent. solution of gum or a 6-per-cent. solution of gelatin. As it happens, the viscosity of such solutions is only very little higher than that of blood. If stronger solutions are used, water is attracted from the tissues and the blood is diluted. This was actually found to be the case when 8-per-cent. gelatin was injected. The viscosity of a sample of blood taken immediately after the injection was 4·2 times that of water; after an hour the viscosity had decreased to 3·4 times that of water.

As already pointed out, with equal cardiac output, the rate of blood flow through the organs remains the same, although the arterial pressure may be higher, if this rise of arterial pressure is due to increased peripheral resistance from rise of viscosity. It might be thought that increased viscosity is not desirable, since the work of the heart is increased thereby. In practice, however, the output of the heart falls with a low blood pressure, partly owing to insufficient inflow from the veins, partly owing to the heart muscle suffering from deficient supply of oxygen.

It is desirable, therefore, to increase both the viscosity and the colloidal osmotic pressure of solutions used for intravenous injection after loss of blood. This can be done effectively by the addition of 6 per cent. gelatin or 7 per cent. gum acacia to Ringer's solution.

Loss of Vascular Tone.

We pass on to those cases where the arterial pressure is low on account of vascular dilatation, without diminution of the volume of blood in circulation. This condition was obtained in my experiments either by dividing the spinal cord at the foramen magnum, or by decapitation in Sherrington's manner. It was found that, although solutions containing gum or gelatin

were more effective than pure Ringer's solution, the rise in arterial pressure did not remain at any considerably raised level for more than 5 mins. or so. It appears from Boycott's experiments (11) that increase in the total volume of blood, at least in rabbits, is apt to lead to heart failure, presumably from over-distension. The cat's heart does not so readily suffer in this way, and it is to be expected that a rise in venous pressure would be found to occur if the falling blood pressure were due to this cause. I have made several observations by connecting a cannula in the vena cava end of the renal vein with a small indiarubber recording tambour filled with half-saturated sodium sulphate solution. The results obtained were rather contrary to my expectation, in that signs of heart failure were not obvious. For example, in a small cat, the arterial pressure after section of the cord was 40 mm. of mercury, and the venous pressure 47 to 50 mm. of sodium sulphate solution of a density of 1·047. The injection of 50 c.c. of Ringer's solution containing 6 per cent. of gum and 3 per cent. of soluble starch raised the arterial pressure to 110 mm. of mercury, but it fell again in 18 mins. to 80 mm. The immediate effect of the injection was to raise the venous pressure to 100 mm. of Na_2SO_4. But, as the arterial pressure fell, the venous pressure also fell along with it, and when the former was 80 mm. of mercury the latter had returned to its original value. Moreover, the heart beats were no smaller than the initial ones, and even greater than those before the injection of the gum. In experiments in which a membrane manometer was used to record the arterial pressure, it was clear that the fall of blood pressure was not accompanied by any decrease in the vigour of the cardiac contractions. On two occasions on which œdema of the lungs came on in the course of the experiment, a rise in venous pressure up to 160 mm. or more of sodium sulphate solution occurred, as would be expected. In the spinal cat, the rise of arterial pressure produced by injection of gum solutions appears to last for a shorter time than when the cord is merely cut. But here again, although the pressure may have fallen to its original value, the heart beats were larger. There was no considerable rise of venous pressure, but by repetition of injections a permanent rise in it could be produced, and sooner or later heart failure came on. The general impression one obtains from these experiments is that the failure of gum injections to maintain for any considerable length of time the temporary rise of blood pressure is not to be accounted for by heart failure resulting from over-distension of the vascular system.

Some experiments were made to discover whether an increase of the viscosity of the blood, without increasing its volume, would be more effective. This was done by removing a portion of the blood, defibrinating, centrifuging,

and suspending the deposit of corpuscles in a sufficient volume of 7-per-cent. gum solution to make up the volume removed. Since, as Table II shows, the viscosity of the blood is mainly due to the corpuscles, such a solution as that mentioned would have a considerably greater viscosity than that of the blood, because that of the gum solution alone is equal to that of blood. It is somewhat surprising, however, that these solutions of high viscosity have no better effect than those whose viscosity is no higher than that of blood. It is possible that the heart may be overloaded, although the venous pressure did not rise higher than 36 mm. of sodium sulphate solution. Even after 50 c.c. more gum solution and 25 c.c. of saline, the venous pressure was only 60 to 70 mm. of sodium sulphate solution, although in asphyxia it rose to 100 mm. We may call to mind the experiments of Evans and Ogawa (13), in which it was found that the output of the heart-lung preparation was decreased by increase in viscosity of the blood above the normal value. These observers hold that the effect is mainly due to increased resistance to inflow through the tubes supplying the heart. The practical point is that no advantage is to be gained by increasing the viscosity of the blood above its normal value.

This statement applies also where the blood pressure was low on account of hæmorrhage. If the viscosity of the fluid run in to replace the loss was increased by the addition of corpuscles from centrifuged blood, the rise of pressure was no greater than if the solution had only the normal viscosity of blood. It is probable that the accommodation mechanism to be referred to below comes into play under such circumstances.

My experiments fail to supply an answer to the question why the effect of intravenous injections, even of gum solutions, is so much less lasting when the low pressure is due to vaso-dilatation than it is when due to loss of blood.

Since the fall of pressure in the former case is due to arterial dilatation, it is natural to test whether the administration of a substance which causes vaso-constriction, such as adrenaline, is what is needed. But the effect of adrenaline is very transitory, so that it would be necessary to give it continuously or in repeated doses. Barium chloride is advocated by Langley (14), and has a more prolonged action. I am able to confirm its value. In doses of 2 mgrm. per kilogramme of body weight in the cat, it does not affect vaso-motor reflexes, and produces a large and prolonged increase in arterial pressure. It may, with advantage, be combined with a moderate amount of gum solution, and given in 1 mgrm. doses, as the following experiment shows:—

Cat of 1·25 kgrm. weight. Cord cut at foramen magnum and vagi cut.

Arterial pressure 41 mm. of mercury. Heart beats very small. Three cubic centimetres of 0·04-per-cent. barium chloride given intravenously (=1 mgrm. per kilogramme). Pressure raised to 63 mm. Twenty cubic centimetres of gum solution were then given. The pressure was raised to 96 mm. It remained high for 7 or 8 minutes, but slowly fell, until in 27 minutes it was 54 mm. A further dose of barium chloride did not raise the pressure much. The vaso-constrictor mechanism was found finally to respond to asphyxial stimulation.

It appears that in physiological experiments the injection of barium chloride combined with gum solution will be found useful. Whether it would be admissible for clinical use, I am unable to state.

Accommodation of the Vascular System.

Some incidental observations made in the course of the preceding research are of interest.

It has long been known that after hæmorrhage there is a gradual rise of blood pressure, and also that an artificially produced rise of pressure causes a reflex vaso-dilatation. The latter is generally regarded as due to stimulation of receptor endings of the depressor nerves. Heidenhain appears to have thought that the vasomotor centres are directly sensitive to rise and fall of arterial pressure. But very little direct evidence has been obtained towards solution of the problem. Johansson and Tigerstedt (15) state that loss of blood results in vaso-constriction, but I have been unable to find the experimental evidence in their paper.

Clearly the only way in which direct evidence can be obtained is by observations on the state of the arterioles in an organ whose circulation is independent of that of the animal to which it belongs, while it is still in nervous connection therewith. Experiments of this kind have been made by Pilcher and Sollmann (16) on artificially perfused spleens. They found that hæmorrhage caused vaso-constriction, transfusion of blood caused vaso-dilatation.

The question seemed of sufficient importance to warrant further experiments, and on another organ. I chose the hind leg of the cat. The whole of the tissues, excluding the femoral artery and vein and the nerves, were tied off by a series of strong ligatures. As perfusion fluid, 7-per-cent. gum in Ringer's solution was used. This was aërated by blowing air through it and was driven through the limb under constant pressure by means of a small pump. A side outlet on the delivery side served to keep the pressure constant and was adjusted by means of a screw pinchcock. The pressure was also controlled by a manometer attached to the cannula in the femoral artery.

The rate of blood flow through the limb was recorded by an electrical drop recorder.

It has been already mentioned that, after blood has been removed, the arterial pressure rises again fairly rapidly to a certain degree, independently of the injection of any fluid. This rise appears to be too rapid to be accounted for, at all events in its initial stage, by absorption of fluid from the tissues. Indeed, I was unable to obtain evidence of dilution of the blood within 3 mins., although the arterial pressure had risen from 38 mm. of mercury to 66 mm. When the circulation through the perfused limb was observed during this period, there was seen to be a progressive slowing of the rate of flow, this slowing was replaced by an increased rate when injections of gum solutions were made into the jugular vein of the animal itself. The results of Pilcher and Sollmann were thus confirmed. It was noticed also, as would be expected, that the first stage of asphyxia was accompanied by a slowing of the circulation through the limb. The constriction passed off in the later stages, as the nerve centres become paralysed.

We see now why it was found in some cases that injection of gum solution, of equal volume and viscosity to that of the blood removed, caused a temporary rise of arterial pressure above that existing before the loss of blood. The injection was made into a system in which the arterioles were to some extent constricted by impulses from nerve centres.

The cause of the peripheral vaso-dilatation produced by rise of arterial pressure seems clear. The depressor nerves were intact, and a sufficient explanation is afforded by stimulation of their receptor endings in the aorta or heart. The peripheral vaso-constriction produced by fall of pressure is not quite so simply explained. Pilcher and Sollmann are inclined to attribute it to anæmia of the vaso-constrictor centre. The result of this is presumably a greater or less accumulation of carbon dioxide, not sufficiently rapidly removed by the blood current, and it has been shown that carbon dioxide excites nerve centres. I do not altogether understand whether these authors regard this as the cause. They state that they have excluded asphyxial effects by the insufflation of oxygen. But it does not follow that more oxygen is actually supplied to the nerve centres, since the blood may have been practically saturated already, and the rate of blood flow is not necessarily increased. There may, therefore, have been a slight rise in the carbon dioxide content of the centres, even when oxygen is insufflated into the lungs. The comparatively slow rate of onset of the vaso-constriction in my experiments is in favour of this explanation. A brief stimulation of the vagus nerve, so that the heart was stopped, did not result in any detectable change in the rate of flow through the limb.

Summary.

When the arterial pressure is low from loss of blood, it cannot be brought back, except to a certain degree, by the injection of saline solution in volume equal to that of the blood lost. But if the viscosity of such solutions is raised to that of the blood, a return to normal height is possible.

The effect of saline injections is also much less lasting than that of solutions containing gum or gelatin. The difference in this case is due to the osmotic pressure of the colloids, by which loss of water by the kidneys and to the tissues is prevented. Solutions containing gum do not produce œdema in artificial perfusion of organs.

When the fall of blood pressure is due to peripheral vaso-dilatation, gum or gelatin solutions, although more effective than pure saline, produce a much less permanent rise than in cases of loss of blood. No signs of heart failure could be detected and the cause of the fall of the raised pressure to its original height is still obscure. The combination of a small dose of barium chloride, as recommended by Langley, with a moderate amount of gum solution was found to be the most satisfactory method in such cases and no diminution of vaso-motor excitability resulted.

The view that fall of arterial pressure produces peripheral vaso-constriction by means of nervous channels and that rise of arterial pressure produces vaso-dilatation was confirmed by artificial perfusion of a limb.

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Man's Mechanical Efficiency in Work Performance and the Cost of the Movements Involved (Treated Separately).

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Many of the data contained in this paper have been already published* and submitted to a preliminary process of analysis. From the arrangement then made it was seen that the body-weight exercised two separate, and opposing, influences on the heat production associated with muscular work. A certain steady rate of movement was maintained throughout a long series of experiments, and this was complicated to a different degree, in different groups of experiments, with the performance of different, increasing, amounts of mechanical work. When the heat production was comparatively small, in the case of minimal work performance, it was observed to vary directly with the body-weights of the individual subjects. On the other hand, when larger, this variation was less noticeable, and at a certain stage of increase in the performance of work it was found to have disappeared completely. The fact was very definite, so that in four different groups of experiments arranged in order of reference to rising values of mechanical work the total heat productions measured varied in Group A directly with $W^{4/3}$, in Group B with $W^{2/3}$, in Group C with $W^{1/3}$, and in Group D with W^{0} (*loc. cit.*, p. 111). No attempt was made at the time, other than contained in a statement of suggestions requiring consideration, to explain this phenomenon, for which course, indeed, an excuse might be found in the labour involved in collecting the information, and the even greater labour of dealing similarly with the very extensive series of measurements underlying the published data. To this problem, then, attention is once more directed in the present paper. In the meantime, these original data have been elaborately and excellently examined by Glazebrook and Dye† in a manner meriting very considerable interest.

Before once more encountering these facts, an explanation of the chief terms utilised may be of advantage, since the mode of experiment and the actual measurements have of necessity to be kept out of sight, and no opportunities arise therefore for an observation of the way in which the measurements are summed to form the total data displayed. Thus, for

* "Studies in the Heat-production Associated with Muscular Work.—Preliminary Communication," 'Roy. Soc. Proc.,' B, vol. 87, p. 96 *et seq.* (1913).

† 'Roy. Soc. Proc.,' B, vol. 87, p. 311 *et seq.* (1914).

example, the main data, throughout termed "heat productions," include frequently a larger quantity of heat than that dissipated from the experimental subject as such, since they include an allowance made for any additional heat stored in his body (an allowance assessed with reference to the rectal temperature), and also include the heat dissipated from the experimental machine (cycle) whenever, and to the same extent as, work is performed upon it by the subject. It is clear that only such sums of the total transformation of energy by the subject are of major physiological interest, as alone equivalent to data obtained from examinations of the exchange of oxygen and carbon dioxide in the concomitant process of respiration, and to data obtained in any other fashion as to the oxidation of material in the body.

Then, again, it is necessary to define the usage of the term "efficiency," since although at the outset of these experiments that usage was as far as possible defined by the very nature of the experiments, occasion has since arisen to utilise the term in an unanticipated way. Thus, originally,* they were arranged to provide a determination of "efficiency" by the comparison of increments in work performance with associated increments of heat production, and that arrangement is very definitely continued in the data under discussion, since the process of experiment was narrowed down to an examination of numbers of individual subjects in two groups of experiments (A and C), differing only from one another by an increment of work and its consequences. Owing to an accident these groups were interrupted, and later, after a complete overhauling of the calorimeter and its apparatus, under improved conditions the same process was renewed, but the results of this later series have been classified under Groups B and D. In either case it was intended to deal finally with the question of efficiency by a comparison of increments observed under similar experimental conditions and it is to be dealt with best by making that comparison where those conditions were at their best, that is, in the later pair of groups (B and D). Utilised in reference to such a comparison, there is not much chance that any misunderstanding can arise as to the meaning of "efficiency." When later the usage of the term is expanded, as first when the efficiency prevalent in the performance of the whole of the external work is considered (as distinguished from the increment) in each of the experiments of Group B and of Group D, even at that stage the term will probably not be misunderstood, since it will be clear that the work done is then being compared to that fraction of the heat production which includes it, and which is associated with its performance and with nothing else, not even

* 'Brit. Assoc. Reports,' p. 289 (1912).

with the accomplishment of the movement in the course of which it is performed. So far the use is simple, and may be expressed in symbols as follows: Let K equal the work done, and $x+\mathrm{K}$ the heat production, the incremental efficiency is the ratio $d\mathrm{K}/d(x+\mathrm{K})$. When the total efficiency of work performance is dealt with, then x must be considered as split up into fractions $y+z$, where z is equal to $\phi\mathrm{K}$, and where y is "everything else" that has no relation to K, in this case the ratio is $\mathrm{K}/(\phi\mathrm{K}+\mathrm{K})$. When later "everything else" is dealt with, some simplification ensues, since for the purposes of this paper—for reasons better explained in the conclusion, when the results obtained by such a method of procedure have been examined and found in order—"everything else" which is left of the heat production when $\phi\mathrm{K}$ has been subtracted is considered in its entirety as the "cost of movement," no attempt whatever being made to treat it as if again analysable into fractions such as are required by the conventional view that a part of it is "resting metabolism," and only the remainder associated with movement. However, it is in this latter case, in reference to movement, most difficult to estimate the prevalent efficiency, because of the impossibility of directly measuring the work done in movement, but it will be seen that there is promise that an analysis of the "cost of movement"

Data of Groups B and D reprinted in Abbreviated Form from the Preliminary Communication. *

	Date, 1913.	"Stripped weight."	Revs. per minute.	Name.	Heat production in kalories per hour.
Group B.—Standard rate of movement 60 revs. per minute, associated work 19 kals. per hour.					
I	January 28, 29; February 3, 4, 5	54·6	60	Bennett	193
II	February 17	62·1	59·8	Kemp	218
III	March 3	50·3	60	Gamm	197
IV	March 4	60·5	60	Rae	212
V	March 5	43·7	60	Armstrong	177
Group D.—Standard rate 60 revs. per min., associated work 56 kals. per hour.					
I	February 18, 26	62·1	60·3	Kemp	350
II	January 27, 30, 31; February 13, 19	54·6	59·8	Bennett	335
III	February 20	60·5	60·4	Rae	347
IV	February 21	60·4	60·5	Hill	346
V	February 24	68·3	60·6	Sharrard	352
VI	February 25, 28	43·7	60·4	Armstrong	346

* 'Roy. Soc. Proc.,' *loc. cit.*, pp. 108, 109.

may somewhat unexpectedly disintegrate that quantity into terms of work done and price paid.

Turning then to the data of the two more recent groups of experiments, B and D (*loc. cit.*, pp. 108, 109), it will be seen that four names, and not more than four, are to be found in both groups, and so provide four individual opportunities for a determination of efficiency by reference to increments of work and heat production. Always speaking of these subjects, Kemp, Rae, Bennett, Armstrong, in the same order, they may be thought of as differing from one another in several ways which seemed to have so little influence on the data that they were not mentioned in the process of preliminary analysis. Thus their heights are different, respectively 168·7, 171·8, 171·2, and 156·4 cm. Their "figures" are different, as may be deduced from a comparison of these heights with the cube roots of their "stripped weights," their respective heights in these terms being, 4·26 $W^{1/3}$, 4·37 $W^{1/3}$, 4·50 $W^{1/3}$, and 4·44 $W^{1/3}$; from which it may be inferred that Bennett was slender (4·5 $W^{1/3}$), whereas Kemp was sturdy (4·26 $W^{1/3}$), and the other two were of intermediate types. The clothes worn weighed respectively, 1·5, 1·0, 3·7, and 3·8 kgrm.; the lighter clothes being the "athletic exercise" garb of two medical students, the heavier the ordinary clothes of two junior laboratory assistants. As a matter of fact, it is difficult to control clothing and the attempt made was limited to the supervision, and provision where necessary, of light shoes. Then the mean rectal temperatures during that part of the experiments (second hour) covered by the data were respectively, 36·6, 37·3, 37·0, and 37·7° C.; the mean surface temperatures, 29·8, 29·9, 31·8, and 34·0° C. More differences, of less importance, could also be mentioned in terms of age, diet, and habit.

First, taking the data from the Tables without correction, that is to say, dealing with all four subjects as if they had each performed exactly the standard amount of work required, equivalent to 56 kalories in D, to 19 kalories in B, and therefore providing an increment of 37 kalories: this increment is then compared with the measured increments of heat production, which differ in the individual cases.

Name.	Weight "stripped."	Heat production.		Increment.	Factors of increment.
		Group D.	Group B.		
	kgrm.				
(1) Kemp	62·1	350	128	132	37 × 3·57
(2) Rae	60·5	347	212	135	37 × 3·65
(3) Bennett	54·6	335	193	142	37 × 3·84
(4) Armstrong	48·7	346	177	169	37 × 4·57

It will be seen that the respective ratios of the number 37 to the numbers 132, 135, 142 and 169 are the individual values of F the efficiency, and realised that the individual values of E (the reciprocal of the "efficiency in work performance") are contained in the last column in the factors 3·57, 3·65, 3·84, and 4·57. The values of these factors expressed in terms of the "stripped weights" of the different subjects are:—

(1) Kemp .... $E = 67\ W^{-0\cdot711}$,

(2) Rae .... $E = 67\ W^{-0\cdot710}$,

(3) Bennett .... $E = 67\ W^{-0\cdot715}$,

(4) Armstrong .... $E = 67\ W^{-0\cdot711}$.

It is of advantage to display these figures in this way prior to making the necessary corrections, since the method of correction is dependent on the accuracy of separate experiments in which measurements were made of the power absorbed by a motor (1) when driving the cycle against the particular brake used in the experiments, and (2), always in the same series of observations, against rope-brakes arranged to entail the same power-absorption. In these experiments it was accurately ascertained that in the neighbourhood of the experimental rate, the power-absorption, and therefore the work done, in the cycling experiments varied with the square of the rate. Aberrations from the standard rate therefore must be regarded as seriously affecting the amount of work done.

Examining the aberrations in rate shown in the tabulated data (Groups B and D, *loc. cit.*), it is found that, with the exception of Bennett, the subjects deviated sensibly and in much the same proportion from this rate in Group D, but maintained it steadily in Group B. The explanation is simple: conducted by the same rhythmical light signal, they were dependent on the clock from which the signal was worked (electrical contact), except Bennett, who preferred to be guided by the tick of a clock inside the calorimeter. These aberrations have been corrected for, in every single datum in the tabulated data, on the precise basis of variation with the square of the rate, and the average correction so obtained entails the following alterations in the statement of work-performance:—

Name.	Actual work, K.		Actual increment, dK.
	K_1, Group B.	K_2, Group D.	
(1) Kemp	18·87	56·56	37·69
(2) Rae	19·00	56·76	37·76
(3) Bennett	19·00	55·59	36·59
(4) Armstrong	19·00	56·67	37·67

As a consequence the values of E formerly given must be altered from (1) 3·57, (2) 3·65, (3) 3·84, (4) 4·57, to (1) 3·50, (2) 3·58, (3) 3·88, (4) 4·49, and the efficiencies prevalent in the four different cases are: (1) 28·6, (2) 28·0, (3) 25·8, and (4) 22·3 per cent. respectively.

Expressed in terms of the "stripped weights" of the different subjects the observed values of E are as follows:—

(1) Kemp .... $64{\cdot}57\ W^{-0{\cdot}708}$,
(2) Rae .... $64{\cdot}57\ W^{-0{\cdot}703}$,
(3) Bennett .... $64{\cdot}57\ W^{-0{\cdot}703}$,
(4) Armstrong .... $64{\cdot}57\ W^{-0{\cdot}708}$.

It is therefore clear that efficiency in work-performance is dominated by the value of the body-weight, that it is greater with the greater weight, and therefore that on this account greater weight is an advantage, diminishing heat production. In the particular case just examined it is found, in fact, that

$$F = W^{0{\cdot}705}/64{\cdot}6.$$

However, it is essential, when making this statement, that some indication should be inserted of my opinion that this expression covers only a particular problem and refers, in unmodified form, only to work upon a particular machine. I shall, therefore, venture to insert in the statement a particular function of the body weight, $1/P$ (see (*l*), p. 407), which is of the average value of $W^{0{\cdot}208}/4{\cdot}04$ in these particular subjects, and has a definitely particular average value in this particular case. Modified by its insertion, the efficiency statement is, therefore, as follows:—

$$F = W^{\frac{1}{2}}/16\,P.$$

NOTE.—It is of interest that there is some slight evidence in these data of the influence of factors of secondary importance. Thus in the data of Group D ('Roy. Soc. Proc.,' *loc. cit.*, p. 109) five separate experiments are recorded on Bennett in which his rates of cycling were respectively 59·7, 60·0, 60·1, 59·0, and 60·0. Notwithstanding these differences in rate of movement, and the still larger consequences which ensue in the rate of work performance on the brake (see above), the respective heat-productions are recorded as 338, 332, 336, 333, and 338. Thus, more especially attending to the fourth of these experiments, in which the rate of cycling fell to an annoying degree, Bennett's temperature may explain the fact that the heat production did not similarly fall. At the time he maintained that the cycle-counter was at fault, and that as a fact the rate was properly continued, but his record differs on that day from every other day in the following important points: (1) initial temperature 37·5° C., replacing average 37·1° C.; (2) rectal temperature during experiment 37·33° C. instead of 36·8° C.; (3) surface temperature 33·65° C. instead of 30·81° C., and consequently (*d*) difference of level between rectal and surface temperatures 3·7° C. instead of 6·0° C. In short—and not as an explanation—on that occasion Bennett suffered from, and complained of, a heavy cold. Somewhat similar pathological interest is to be attached to the experiment on Sharrard in the same group.

Up to the present nothing but the increments have been examined, and it is of interest now to observe what order can be obtained on the assumption that this efficiency prevailed similarly in the total performance of external work. Subtracting then the quantity EK_1 from the experiments in Group B, and EK_2 from those in Group D, in both cases utilising the now observed individual values of E and the corrected values of K_1 and K_2, the following residues are obtained.

Residues or Costs of Movement.

Name.	Group B.	Group D.
(1) Kemp	151·9	152·0
(2) Rae	144·1	144·2
(3) Bennett	119·2	119·6
(4) Armstrong	91·5	91·7

Although the slightly greater rate of movement (603/600) in Group D might have been expected to produce somewhat larger consequences, to which Bennett should have proved an exception, yet the figures are very satisfactory evidence of the comparability of the two groups of experiments. In view of this evidence these data are accepted as entities separable from the total heat production, and as representing the cost of the underlying movement. Before dealing with them more precisely certain additional data are introduced.

Additional Data (Briscoe).

Doubtless some of the accuracy of the experiments just quoted is to be assigned to the monotonous repetitions of similar experiments, every experiment being a "drill" in handling the very complicated apparatus required, in precisely the most convenient way. The series of experiments now quoted from were of a different type, since large variations in heat production were measured in successive experiments. No doubt they suffer to some slight degree from that fact. Then again they were not all of precisely the same duration, some longer and some shorter than those of the "efficiency groups"; and in addition the calibration of the work done on the cycle was not so satisfactory, nor had it the same direct relation to the actual experimental rates of movement, since in several experiments the subject initiated and maintained his own rate. Once that is said, however, in other points they were similar. The rate of cycling was maintained the same, however much it was varied in different experiments, throughout the whole time of each single experiment. The sets of observations were made at the end of each five-minute period, and none are reckoned in the data that were

taken in the first hour. The "accountancy" of measurements has followed exactly the same rigid plan, and the data now published have been carefully scrutinised again on exactly similar lines. The subject's efficiency, as calculated from his own results, and in agreement with those already considered, is such that the subtraction necessary to remove the moiety of heat production associated with external work performance is 3·85 K. Removing these fractions the "cost of movement" is left, and in this case it represents that cost at definitely different rates of movement.

E. J. Briscoe, 1912. "Stripped weight" 55·8 kgrm. E = 3·85.

Date.	V. Revs. per minute.	K. Work.	H. Heat production.	EK. Subtraction.	Q = H − EK. Residue.
1912.					
(1) May 24.........	40	21	159	81	79
*(2) May 14.........	60	13	167	50	117
*(3) May 17.........	60	26	212	100	112
*(4) May 20.........	60	34·5	244	133	111
*(5) May 22.........	60	42·5	286	164	122
(6) May 7.........	72	49	341	189	152
April 25	74	16	222	62	160
May 21.........	80	73	456	281	175
†March 28......	97 (?)	10	291	40	251
†March 29......	98 (?)	11	316	42	274

* Data in British Association Report, 1912, p. 289, numbered there as 2, 3, 4, 5.

† The query by the side of the revolution rate will be understood to refer to the difficulty of maintaining these fast rates in a perfectly uniform way, there always being a tendency for groups of faster to succeed groups of slower movements.

These Briscoe data have been given in the same form as all the data up to the present, and may be compared at once with them, but for the purposes of the later part of this paper they are now changed in form. Kalories per hour are now changed into small calories per second, revolutions per minute into "per second," and doubling this revolution rate, so taking account of the two complete leg-movements associated with each revolution of the cycle pedals, they are presented as "strides per second."

Another change may also be noticed from this point onwards, namely, the large number of apparently significant figures in which the rates of movement are expressed (thus, 2·667 per sec.), but this method has proved of value, in so far as these figures are submitted to speculative arrangement always in the same definite form in which they are given.

Table I.—Briscoe.

V = strides per second. Q = residue or "cost of movement."

V	1·333	2·000	2·400	2·466	2·667	3·200	3·267
log V	0·124	0·301	0·380	0·392	0·426	0·505	0·514
Q	22	32	42	44	49	70	76
log Q	1·342	1·505	1·623	1·643	1·690	1·845	1·881

Further Additional Data (Douglas).

To complete the range of data essential to a full consideration of the "cost of movement," I have, of necessity, had to consult the data given by other investigators, and have chosen as most suitable for my purpose equivalent data published by Douglas and Haldane,* which are in their original form measurements of the oxygen absorbed and the carbon dioxide produced by Douglas when walking on the grass at Oxford at rates varied from 2 to 5 miles per hour. These data have been converted into calories per second, following the Zuntz and Schumburg method in exact detail. The method may be readily ascertained from Benedict and Cathcart's description.† This done, I have converted "miles per hour" into "strides per second" in an arbitrary fashion, since the stride-length of Douglas is not given. For this purpose I have credited him with a length of stride of 0·837 metre (33 inches), thus allowing 0·533 stride per sec. as the equivalent of 1 mile per hour.

In dealing with these data no subtraction whatever has been made. The view is taken that the process of "walking on the grass at Oxford" is not associated with any other than a negligible amount of external work performance. The whole heat production is thus in this case treated as "cost of movement," and is dealt with as if caused by phenomena completely resembling those underlying the residue of heat production in Briscoe's case, completely, that is to say in everything but magnitude.

Table II.—Douglas Walking on the Grass at Oxford.

V = strides per second. H = heat production in calories per second.

V	2·667	2·400	2·133	1·600	1·067
log V	0·426	0·380	0·329	0·204	0·028
H	210	164	131	88·5	63
log H	2·322	2·215	2·118	1·947	1·799

* 'Journ. Physiol.,' vol. 45, p. 235 *et seq.* (1912); also quoted in 'Phil. Trans.,' B, vol. 203.

† 'Muscular Work—A Metabolic Study, with Special Reference to the Efficiency of the Human Body as a Machine,' Carnegie Institution, Washington, 1913, p. 33.

The Cost of Movement.

Formulation of the Cost.

It is a matter of common knowledge that the "cost of movement" increases with increasing body weight. It is therefore different from the cost of work performance, for which heavier individuals are chosen for the continued performance of heavy work. That the fact is a very definite one may be seen at once from the residues of my four cycling subjects on p. 401. For brevity those residues (Group B) are at once expressed in terms of the individual "stripped weights" of the four subjects subsequent to conversion from kalories per hour into small calories per second (division by 3·6).

(1) Kemp .... $Q = 0{\cdot}1114\ W^{1\cdot438}$,
(2) Rae .... $Q = 0{\cdot}1096\ W^{1\cdot438}$,
(3) Bennett .... $Q = 0{\cdot}1053\ W^{1\cdot438}$,
(4) Armstrong .... $Q = 0{\cdot}1114\ W^{1\cdot438}$,

and at the same rate of movement (see Table I, 2 strides per second),

(5) Briscoe .... $Q = 0{\cdot}0991\ W^{1\cdot438}$.

Taking the average of the first four, obtained under the same experimental conditions, it may be said that at this rate of movement (one cycle revolution, or two strides per second),

$$Q = 0{\cdot}1094\ W^{1\cdot438}. \qquad (a)$$

And now turning to the influence of the rate of movement on this cost, it is also certainly very definite, even if complex. Thus the whole of the Douglas data (Table II) may reasonably be considered as expressible in the following formula:—

$$H = 52{\cdot}37\ (1{\cdot}475\ V)^{0\cdot380\,V}, \qquad (b)$$

as is shown by a comparison of the data,

(1) 210, 164, 131, 88·5, 63,

with the values deduced from the formula,

(2) 210, 166, 133, 88, 63.

As a matter of fact, it is possible to make a choice between this formula and others of a somewhat similar type, equally, if not more, satisfactory for this purpose, but this formula has been deliberately chosen as of a certain greater rigidity of type which is of value when comparisions are made with attempts to formulate the cost of other movements. Thus, for example, the whole of the Briscoe data can be reasonably held to be expressible in an exactly similar, and similarly rigid, formula as follows:—

$$Q = 16{\cdot}45\ (1{\cdot}783\ V)^{0\cdot260\,V}, \qquad (c)$$

as is shown by a comparison of the data

(1) 22, 32, 42, 44, 49, 70, 76

with the values deduced from the formula

(2) 22, 32, 41, 42, 48, 70, 73.

The agreement is certainly least where the experimental data are necessarily fallible.

In reference to the influence of "rate" upon the cost of movement, and in exact explanation of the nature of the formulæ, it may be said at once that the point of greatest importance lies in the fact that the cost per stride is least at a certain intermediate rate of movement, and therefore also the cost of progression is least at the same intermediate rate. In both of these formulæ that fact is placed in unusual prominence by the direct insertion of this economical rate in a definite position of importance in the formulæ. The cost per stride in Douglas' case, that is to say, the value of HV^{-1}, is least when V has the value 1·475, and in Briscoe's case the value 1·783. Speaking of the particular value of V as in each case P, then the two formulæ have the resemblance shown below:—

(*b*) Douglas .... $H = 52{\cdot}37\,(PV)^{0{\cdot}380\,V}$,
(*c*) Briscoe .... $Q = 16{\cdot}45\,(PV)^{0{\cdot}260\,V}$.

Nor is this the end of the resemblance, as may be seen from the considerations stated below.

Digressing a moment, but as briefly as possible, it may be stated as an axiom that, with regard to every formula of the general type, $R = x\,(yV)^{zV}$, the value of V at which RV^{-1} is minimal is determined by the relation

$$z\,V_1\,(\log_e V_1 + \log_e y + 1) = 1.$$

In this particular case $P = V_1 = y$, and therefore

$$zP\,(\log_e P + \log_e P + 1) = 1,$$

therefore

$$z = 1/[P\,(2\log_e P + 1)]. \qquad (d)$$

That is to say that, in this particular rigid formula, z is also a function of P, and may be represented by P′; and this is exactly true in the two formulæ given: in the one case 0·380 has this relation to 1·475, and in the other 0·260 to 1·783. The present resemblance between the two formulæ is therefore seen in the fact they may be both written as follows:—

(*b*) Douglas .... $H = 52{\cdot}37\,(PV)^{P'V}$,
(*c*) Briscoe .... $Q = 16{\cdot}45\,(PV)^{P'V}$.

If it could be shown, then, that 52·37 in the one case, or, as it may be termed, T in formula (*b*), is also a function of 1·475, and the same

function as is 16·45, the T in formula (*c*), of 1·783, then H would be a function of P, Q also a function of P, and the functions would be identical in character. This actually seems to be the case, as is shown incidentally in the following method of dealing with the two formulæ, which is of use later in assessing values of P in additional individual cases (see (*l*), p. 407).

(*b*) Douglas $\quad H = T(PV)^{P'V} = 52{\cdot}37\,(1{\cdot}475\,V)^{0{\cdot}380\,V},$

therefore $\quad \log H = 1{\cdot}719 + 0{\cdot}380\,V\log V + 0{\cdot}064\,V,$

$$= 1{\cdot}719\,(1 + 0{\cdot}221\,V\log V) + V\log 1{\cdot}16,$$

therefore $\quad H = 1{\cdot}16^{V}\,T^{1+0{\cdot}221\,V\log V}.$

It will be seen that this new form of statement is permissible, inasmuch as $P^{P'}$ is approximately equal to 1·16, and because

$$P' = 0{\cdot}221 \times 1{\cdot}719 = 0{\cdot}221 \log T. \qquad (e)$$

(*c*) Briscoe $\quad Q = T(PV)^{P'V} = 16{\cdot}45\,(1{\cdot}783)^{0{\cdot}260\,V},$

therefore $\quad \log Q = 1{\cdot}216 + 0{\cdot}260\,V\log V + 0{\cdot}065\,V,$

$$= 1{\cdot}216\,(1 + 0{\cdot}213\,V\log V) + V\log 1{\cdot}16.$$

$$= (1 + 0{\cdot}213\,V\log V)\log T + V\log 1{\cdot}16.$$

therefore $\quad Q = 1{\cdot}16^{V}T^{1+0{\cdot}213\,V\log V}.$

This statement, similar to that given in the case of Douglas, is again also permissible because $P^{P'}$ is approximately equal to 1·16, and because

$$P' = 0{\cdot}213 \times 1{\cdot}216 = 0{\cdot}213 \log T. \qquad (f)$$

Now when (*e*) is compared with (*f*), with an appreciation of the fact that the quantities of heat dealt with in the two cases are in the ratio of 210/49 at the same common value for V, (V = 2·667), also of the ease with which it would be possible to adjust the small difference thus revealed without modifying the formulæ probably in any other way than to make them still more applicable to the data than they are at present, it will be granted that (*e*) and (*f*) reveal T as the same identical function of P. T may then be written as P″. It follows that the formulæ are completely identical in form, and may either be written as follows :—

$$H \text{ or } Q = P''(PV)^{P'V} = \phi(P, V).$$

The only difference, then, between the cost of walking, on the one hand, and the cost of cycling on the other, is to be sought in the different magnitude of P in the two cases, and in either case the cost of movement at every other rate, including that at the "economical rate," where this cost is least, may apparently be anticipated after an examination of the cost at any one definitely maintained rate.

It is now useful to define T in terms of P, and a possible procedure is as follows:—

$$\text{Let } T = 1{\cdot}17\mu, \quad \text{therefore (Douglas) } \mu_1 = 44{\cdot}76, \text{ (Briscoe) } \mu_2 = 14{\cdot}06.$$

$$\text{Let } \mu = yP^z \text{ and therefore } \log\mu = \log y + z\log P,$$

$$\text{therefore (Douglas)} \quad 1{\cdot}651 = \log y + 0{\cdot}169z, \tag{1}$$

$$\text{(Briscoe)} \quad 1{\cdot}148 = \log y + 0{\cdot}251z. \tag{2}$$

From these equations

$$z = -6{\cdot}134, \quad \text{and } \log y = 2{\cdot}687,$$

$$\text{therefore also} \quad y = 486{\cdot}5 = e^{6\cdot187},$$

$$\text{therefore} \quad \mu = e^{6\cdot187}/P^{6\cdot134},$$

$$\text{and} \quad T = 1{\cdot}17\mu = 1{\cdot}17\,e^{6\cdot187}/P^{6\cdot134}. \tag{g}$$

Introducing this expression for T in the general equation

$$H^* = 1{\cdot}17\,(e^{6\cdot187}/P^{6\cdot134})(PV)^{P/V},$$

$$\text{therefore} \quad 0{\cdot}426\,H = \tfrac{1}{2}\,(e^{6\cdot187}/P^{6\cdot134})(PV)^{P/V}. \tag{h}$$

The suggestion implied in the form (*h*) is obvious, and may be briefly expressed by saying that the expression

$$0{\cdot}426H = E'(\tfrac{1}{2}mu^2) = \tfrac{1}{2}(mE')u^2, \tag{j}$$

would represent a rational formula, in which E′ was the reciprocal of the efficiency in movement, also that there is some promise shown in (*h*) of a final statement in this rational form.

Under the impression that this promise is sufficient to permit immediate examination of the formula from such a point of view, I shall venture to speak of part of the formula as the possible "(mE')" factor.

The "(mE′)" Factor.

An attempt to ascertain the relation between $e^{6\cdot187}/P^{6\cdot134}$ and the mass, and at the same time to express $1/P$ in terms of W, may be made very simply by utilising the relation found in the case of the four cycling subjects, $Q = 0{\cdot}1094\ W^{1\cdot438}$. It will be remembered that for both Douglas and Briscoe, and therefore inferentially the cycling subjects, $Q = 1{\cdot}16^V T^{1+\cdot213 V \log V}$. It is true there was a slight difference in Douglas' case, but it was even then of minimal value, and there can be little hesitation in applying the exact form of Briscoe's statement to the subjects examined under similar conditions. In their case, since V = 2, the latter expression becomes $Q = 1{\cdot}346\ T^{1\cdot128}$,

$$0{\cdot}1094W^{1\cdot438} = Q = 1{\cdot}346T^{1\cdot128},$$

* Or Q.

therefore $$T = W^{1\cdot 275}/9\cdot 284 = 1\cdot 17\mu,$$

therefore $$\mu = W^{1\cdot 275}/10\cdot 86. \qquad (k)$$

But

$$\mu = e^{6\cdot 187}/P^{6\cdot 134} = W^{1\cdot 275}/10\cdot 86 \quad \text{therefore} \quad P_c{}^{*} = 4\cdot 042/W^{0\cdot 208}. \qquad (l)$$

Therefore the mE' factor and P have both been expressed in terms of the "stripped weight." However, since the mass in motion includes the clothes, it seems essential at this point to introduce the "clothed weight"; and an interesting way of doing so is to determine the values of P for Kemp and Armstrong, from the general expression (l) given above; and then convert the values so found into terms of the clothed weight. Substituting 62·1 and 43·7 for W in (l), it follows that in Kemp's case $P = 1\cdot 714$, and in Armstrong's case $P = 1\cdot 846$. Now their clothed weights (for weight of clothes, see p. 397) were 63·1 and 47·5 kgrm. respectively; terming this weight W_2, in both cases the given values of P_c may be expressed as

$$P_c = 4\cdot 88/W_2{}^{0\cdot 252}; \qquad (m)$$

substituting this new value for P_c in the "(mE')" factor,

$$e^{6\cdot 187}/P^{6\cdot 134} = (W_2/9\cdot 82)^{1\cdot 546} = (W_2/g)^{1\cdot 546},$$
$$= m^{1\cdot 546},$$

therefore $$mE' = m^{0\cdot 546} \times m. \qquad (n)$$

Recognising now with some certainty the fact that this is indeed a "mass factor," even if qualified by something in the nature of E', it is safe to conclude that either $1/P$ or $1/P^2$ is a unit of length. The conclusion may be said to follow at once from the general cubical nature of the factor, which may, indeed, be described as $(e^2/P^2)^3$ qualified by a correction for clothing (difference between $e^{6\cdot 187}$ and $e^{6\cdot 134}$) and a correction for density (difference between 6·134 and 6·000). Having arrived at this conclusion, however, it is reasonable to be prepared for several different relationships to the whole mass of the body resulting from temporary alterations in this individual unit of length, not only in reference to the individual mass, but also to the degree of shortening or extension of the body mass associated with individual movements. In short, it is reasonable to infer that the comparison between the value of P found for Briscoe and that found for Douglas is not assignable merely to different body weights, but also to an essential distinction between P_c and P_w, the "cycling" and "walking" values of P respectively. Taking this view, I have considered it not unwise to assume that whereas P_c is related

* P_c denotes the "cycling value" of P.

to $W_2^{1/4}$ as shown, it is extremely likely that in the fully extended dimension of the body utilised in walking, P_w will be found rather related to that function of the weight which is of so great comparative value in connection with the linear dimensions of the body, namely, the cube root of the weight. Considering the mass factor, $e^{6\cdot187}P^{-6\cdot134}$, with this possibility in mind, it would seem as if under such circumstances the whole factor would become m^2. This view may be tested immediately, since it should be possible to calculate Douglas' weight on the assumption that in his case $\mu = (W_2/g)^2$.

Thus $\mu = e^{6\cdot187}P^{-6\cdot134} = T/1{\cdot}17 = 44{\cdot}76$ (Douglas),
therefore $44{\cdot}76 = (W_2/g)^2$ and therefore $W_2 = 65{\cdot}6$ kgrm.

But since his weight is given in 1910* as 65 kgrm., the assumption would seem to be reasonably justified. It is probable then that, whereas $P_c = 4{\cdot}88/W_2^{0\cdot252}$, $P_w = 5{\cdot}77/W_2^{0\cdot326}$, so that in Douglas' case the observed value of 1·5 in the case of walking movement would probably correspond with a value of 1·7 in reference to cycling movement.

The Velocity Factor $(PV)^{P'V}$.

In the case of "Douglas walking," it is possible to compare with some interest the square root of the velocity factor on the one hand with the actual horizontal velocity or rate of progression on the other, and perhaps this is best done at first in reference to the most important "economical rate" P, when the cost of stride is least. At the rate P per second, since $V = P$, therefore $(PV)^{P'V} = P^{2PP'} = (1{\cdot}475)^{1\cdot121} = 1{\cdot}546 = (1{\cdot}243)^2$. Again, since the length of a stride is 0·837 metre, the horizontal velocity at the rate P is 0·837 P, and is therefore 1·235 metres per second. For brevity, using the term f in place of $(PV)^{\frac{1}{2}P'V}$, it is seen that there is no great difference at this rate between v and f.

In general, the relation between v and f is such that the line v intersects the curve f at two points, where $V = 1{\cdot}5$ and where $V = 1{\cdot}5^3$; that is to say, that, at rates not very different from P and P^3 respectively, v is equal to f. At intermediate values of V the horizontal velocity is slightly the greater quantity, the maximal difference of 0·213 metre per second being found at the rate $1{\cdot}5^2$, that is to say, at a rate not very different from P^2. Beyond these points on either side of P and P^3 the curve rapidly falls away from the line, so that f becomes much greater than v.

In short, although v is not a tangent to f, yet $v+0{\cdot}213$ is such a tangent at the point where $V = 1{\cdot}5^2$. There does not seem, under these

* 'Journ. Physiol.,' vol. 40, p. 235.

circumstances, any reason to hesitate before suggesting the possibility that f, or $(PV)^{\frac{1}{2}P/V}$, may finally be shown to have some definite relation to the acceleration or sum of accelerations responsible for the quasi-pendular movements from which the horizontal velocity is derived.

As a summary to the sections dealing with mE', and with the velocity factor, it may be stated, then, as not improbable that the empirical formula $0{\cdot}426H = \frac{1}{2}\phi(1/P)(PV)^{P/V}$, may finally be arranged in rational form in some such manner as

$$0{\cdot}426H = \tfrac{1}{2}\,mE'/f^2.$$

Conclusion.

Attention is drawn to the fact that there appears to be some definite order in the heat productions dealt with as "cost of movement" when no allowance is made for synchronous "cost of rest." The order developed by dealing with the facts in this way is significant, in my opinion, of the influence of that control exerted by the central nervous system in arranging the phenomena of a moment to correspond with the requirements of that moment. In my view. "rest" is an entity, and not devoid of a dynamic fraction, "movement" is again an entity of a different type, in which the dynamic fraction is in the forefront, and on this view everything occurring during movement is related to movement. In short, the view is held that there is no "rest" in movement, and apparently with some justification. It is also held, and apparently has indeed been shown, that the cost of movement is identically the same whether the work performed by its means is large or small.

Attention is also drawn to the importance of an "economical rate" in movement as the phenomenon of major interest, and as decided by relationships to bodily dimensions of an exact kind. There is no sign, indeed, that any other circumstances need be considered than mass and length in this connection.

Then, as to the "efficiency" prevalent in the performance of movement, it would seem to *vary inversely* with the mass in motion, but the fact that this is the case suggests at once the conception that this efficiency (so far as it can be examined) is not a genuine efficiency, but is due to the complication of a constant, perhaps, indeed, an absolute efficiency (100 per cent.), by unknown internal resistance directly proportional to the mass engaged in accomplishing visible external work again proportional to the mass, so that the cost varies with the square of the mass.

A similar point is even still more evident with regard to the "efficiency of work performance." In this case a different mechanical problem, leverage

perhaps as contrasted with quasi-pendular movement, exhibits an efficiency *varying directly* with the weight or mass in such a way that the cost is diminished by increase of mass, as if the different mechanical considerations involved in this separable process arranged the mass in the other pan of the scales of cost.

The Typical Form of the Cochlea and its Variations.

By Henry J. Watt.

(Communicated by Prof. D. Noël Paton, F.R.S. Received October 3, 1916.)

The work of this paper is based upon the photographic and descriptive material presented by Dr. A. A. Gray in his two volumes on 'The Labyrinth of Animals,' published by J. and A. Churchill, London, in 1907 and 1908.* I have succeeded in extracting from that impressive mass of material definite results that seem to be of some importance.

The dimensions of the cochlea measured by Dr. Gray are: (1) the diameter of the lowest whorl and (2) of the second whorl ("taken in a plane which passes vertically through the apex of the cochlea and the anterior margin of the round window"); (3) the diameter of the tube of the cochlea in front of the round window; (4) the major axis of the oval window: (5) the slant height of the cochlea ("the distance from the upper margin of the round window to the apex of the organ"); and (6) the number of turns of the cochlea.

I found it desirable to add to these a measurement of the total length of the basilar membrane. That must surely represent more closely and directly than anything else the pitch-range of hearing. Fortunately, a close study of Gray's wonderful photographs showed that an approximate measurement of the length of the basilar membrane (as of the outside edge of the cochlear tube) could be got from them. The symmetrical shape of the cochlea makes it possible to measure the diameters of the successive whorls, no matter from what angle the photograph was taken. (The reader must consult Gray's pictures.) With the help of Gray's measurements of the diameters of the first and second whorls, by close attention to the consistency of these with the dimensions visible in the photograph, and by a careful comparison of the different photographs showing the cochleas of

* *Cf.* also 'Roy. Soc. Proc.,' B, vol. 78, p. 284 ff. (1906), and B, vol. 80, p. 507 ff. (1908).

different animals from the same relative angle, I made what I think is a close approximation to the actual length of the basilar membrane. In this way I constructed a scale drawing of the basilar membrane as seen from above on the axis of the modiolus, and I measured its length with a rotary map measurer. The reader who is not familiar with Gray's photographs will find that they are much more transparent, and that, consequently, much more detail is visible in them, than he might be inclined to expect. Fig. 1 gives, as an example, a difficult case in which Dr. Gray's photograph presents a view of the cochlea from a point on a line at right angles to the axis of the modiolus.

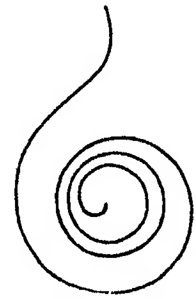

FIG. 1.—(See A. A. Gray: 'The Labyrinth of Animals,' vol. i, Plate XI. — The Tiger.) × $3\frac{1}{2}$ (about).

Doubtless there is a variable error, which probably never exceeded 2 or 3 mm., and was usually much less. And even that maximum is fairly small in comparison with most of the lengths recorded. The small error caused by the rising of the cochlea to an apex I neglected entirely. For a basilar membrane of 52·4 mm. (No. 12) the error neglected is not more than 0·16 mm. For No. 52 the error is 0·1 mm. for a length of 16·3 mm. The results obtained confirm my estimate of the reliability of the measurement of the basilar membrane.

In order to trace the connection between the dimensions of the cochlea and those of the body as a whole, I sought for a measurement which would represent this. I found that one had been recorded by R. Lydekker for most of the species represented in Gray's work, namely, the length of the head and body, not including the tail. For the aquatic mammals I took the length of the whole body. The method is rough, but no other was available, and, under the circumstances, it is probably good enough.

A. *The Typical Form of the Cochlea.*—Table I gives the values of the coefficient of correlation between the various series of measurements, and of the probable error, derived with the use of Karl Pearson's formulæ. I have neglected here any consideration of "the slant height of the cochlea," as that is from its definition much less a matter of the height of the cochlea than of the diameter of the lowest whorl, which is given independently.

1. There is an absolutely certain positive correlation between the diameter of the lowest whorl, on the one hand, and, on the other hand, the diameter of the second whorl, the length of the basilar membrane in the first two whorls, the total length of the basilar membrane, the major axis of the oval window, and the diameter of the tube of the cochlea. These two

last have also a high positive correlation with one another (*vide* Table I, Note 1). The correlation with the length of the head and body is of the same order (*vide* Note 2).

Table I.—The Cochlea of Mammals. Correlation Coefficients (from K. Pearson's formula). 51 or 52 organs (42 for the length of the basilar membrane).

	Diameter of the lowest whorl.	Length of the basilar membrane.	The number of whorls.
Diameter of lowest whorl	—	·954 ± ·009	·088 ± ·092
Diameter of second whorl	·970 ± ·006	·931 ± ·014	·070 ± ·092
Length of the basilar membrane	·963* ± ·008 ·954 ± ·009	—	·155 ± ·101
Major axis of the oval window	·914 ± ·015	·812 ± ·035	·035 ± ·094
Diameter of the tube of cochlea	·806 ± ·033	·760 ± ·044	·195 ± ·090
$\frac{\text{Diameter of second whorl}}{\text{Diameter of first whorl}}$ per cent.	− ·417 ± ·077	− ·349 ± ·091	·000 ± ·094

* For the length of basilar membrane in the first two whorls.—
(1) r for diameter of tube of cochlea to major axis O.W. = ·828± ·030;
(2) r for diameter of lowest whorl to length of head and body = ·864± ·024.

2. The series of correlations for the length of the basilar membrane appears in the second column of the Table. It will be seen that it is parallel in its degrees to the series appearing in the first column, but that the values of the second column, with the exception of the reciprocal pair, are all smaller than those of the first, while the values of the probable error are, of course, larger. If the length of the basilar membrane and the diameter of the lowest whorl were perfectly correlated with one another, this difference between the two columns would mean that my measurement of the length of the basilar membrane is subject to a variable error that is probably greater than the error made by Dr. Gray in his direct measurements, but that is still not disturbing. But, as we shall see, the relative length of the basilar membrane is subject to special variations in relation to the number of whorls of the cochlea (*vide* below, B). These fluctuations would account for some part of the difference between the two columns. In any case, my measurements of the length of the basilar membrane may be taken as good.

3. There is no correlation at all between the number of whorls and the diameter of the lowest, or even of the second, whorl, or the major axis of the oval window, or the percentage relation of the second whorl to the first. The other two correlations, with the diameter of the tube of the cochlea and with the total length of the basilar membrane, are too small to be of any

significance. There is, then, no correlation between the number of whorls and any other dimension of the cochlea.

4. Fig. 2 gives the distribution of the frequency of the different numbers of whorls, *i.e.* there were amongst the 52 organs of mammals measured by

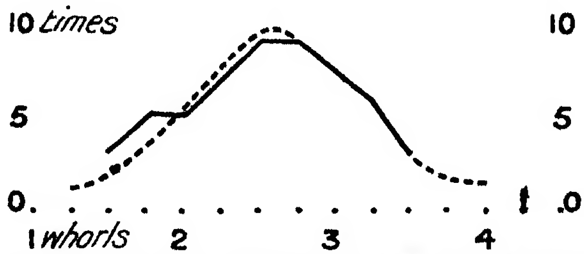

FIG. 2.—Number of Whorls—Distribution of Frequency of each Number.

Dr. Gray, three organs having $1\frac{1}{2}$ whorls, five having $1\frac{3}{4}$ whorls, and so on. One organ had $4\frac{1}{4}$ whorls. The figure shows a striking evenness of distribution about a point between $2\frac{1}{2}$ and $2\frac{3}{4}$ whorls. The average number of whorls of all 52 organs is 2·56 whorls, or a little more than $2\frac{1}{2}$ turns. Thus, the average and the highest frequency agree. We may therefore put down the typical number of whorls of the mammalian cochlea as two and a half.

5. The absence of correlation between the number of whorls and the diameter of the second whorl is interesting. It means that the addition of a third whorl (or of more than one) does not necessitate an expansion of the second whorl to make more room for it. Generally the other whorl is merely added on where the second one stopped without any change in the other dimensions of the cochlea.

6. The two negative correlations of the "ratio of the diameter of the second whorl to that of the first" seem to indicate that the bigger the cochlea is, the greater is its rate of curvature, *i.e.* the smaller is the diameter of the second whorl relatively to that of the first. This correlation, especially as relating to the diameter of the first whorl, approaches towards being a practical certainty. The coefficient is almost $5\frac{1}{2}$ times the amount of the probable error, 6 times yielding practical certainty of correlation.

7. In this connection reference may be made to the only other description applied to the cochlea by Dr. Gray, namely, the distinction of the "flat" from the "sharp" cochlea. In the latter the whorls seem to be piled on the top of one another; in the former the second whorl lies more or less in the coil of the first. This difference is connected with the ratio of the diameter of the second whorl to that of the first. If an arbitrary numerical value be given to Dr. Gray's degrees of flatness and sharpness: very sharp or sharp and

convex, 6; sharp but concave, 5; sharp, 4; medium, 3; flat, 2; very flat, 1; then there is a correlation of $+0{\cdot}482 \pm 0{\cdot}071$ between these values and the ratio of the diameter of the second whorl to that of the first. This correlation is a practical certainty, though not quite decided (*i.e.* $> +0{\cdot}50$). The sharp cochlea has the second whorl relatively wide. A better basis of correlation would probably have been a measurement of the real height of the cochlea, *i.e.* the vertical distance from the level of the under edge of the lowest whorl to the apex of the organ.

We may sum up the data and conclusions thus far by saying that the cochlea is built according to a constant plan, of which the scale alone varies from case to case. This scale shows a decidedly high correlation with the size of the organism as a whole. A change of scale will obviously alter all the dimensions recorded except the number of whorls. But even that number, when it varies independently, does not alter the other dimensions of the cochlea. The only other variant thus far detected is the rate of curvature of the spiral, which is greater in the bigger scale organs.

Fig. 3 shows graphically the relation between the various dimensions of the cochlea on the principle of moving averages. The serial arrangement of the organs follows the increase of the diameter of the lowest whorl. The five lowest values were averaged; then the lowest was omitted and the sixth value in order of size was taken up instead, and the resulting group of five values was averaged; and so on, through the whole series.

8. For the cochlea of birds Gray gives the measurements of 17 organs of different species. The coefficients of correlation between the three series of measurements given, the major axis of the oval window, the diameter of the tube of the cochlea, and the length of the tube (*i.e.* including the lagena), appear in Table II. The values are not so high as those of Table I, but there is a clear correlation between the major axis of the oval window and the length of the tube of the cochlea.

Table II.—The Cochlea of Birds (17 Organs).

	Length of tube of cochlea.	Diameter of tube of cochlea.
Major axis of oval window ...	·754 ± ·070	·600 ± ·104
Diameter of tube of cochlea ...	·508 ± ·121	

For the reptiles and amphibians Gray gives the measurements of only four or five organs. One of these—the black pointed teguixin—is clearly of the avian type. This group is distinguished, as Gray says, only by a shorter cochlear tube. It is too small to be treated statistically.

B. *The Variations in the Form of the Cochlea.*—Having thus shown that the mammalian cochlea is an organ of a definite type, we may enquire whether there is any regularity in the way in which the cochleas of different species deviate from the type. In order to ascertain this I divided each

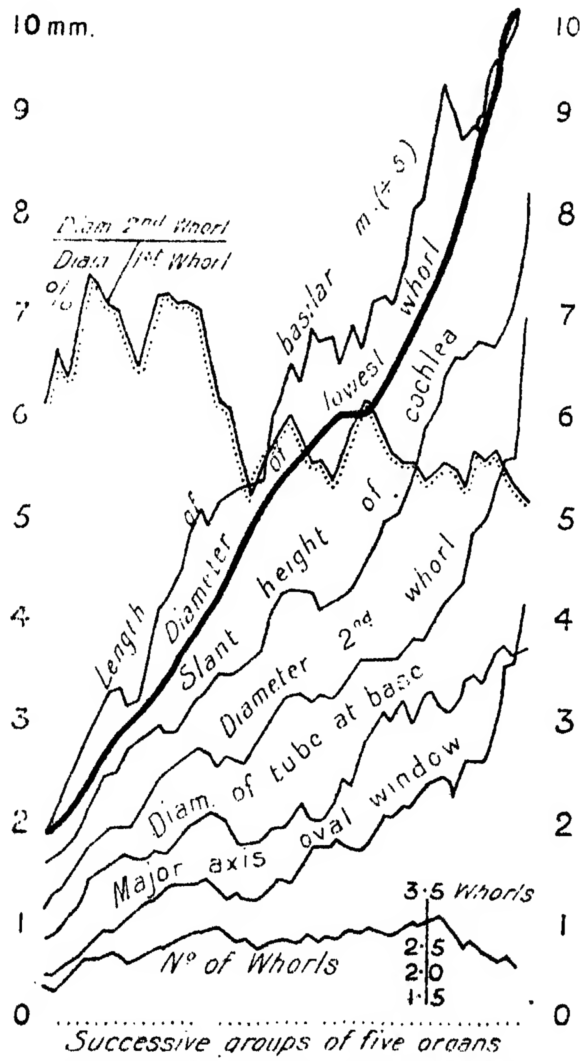

FIG. 3.—Cochlea of Mammals (Moving Averages).

measurement of each organ given by Gray (with the exception of "the slant height of the cochlea") and my estimate of the length of the basilar membrane by the diameter of the lowest whorl of that organ. The resulting values I may call the cochlear indexes of the organ.

9. It may be of interest to state the average index build of the mammalian cochlea; in relation to the diameter of the lowest whorl of the cochlea the proportions are:—

Second whorl	0·61
Diameter of tube in front of round window	0·44
Major axis oval window	0·32
Length of basilar membrane	5·65
Number of whorls (absolute)	2·5 +

In Table III the correlations between the different cochlear indexes are given. The following conclusions may be drawn:—

Table III.—Cochlear Indexes. (Index = any dimension divided by the diameter of the lowest whorl.)

	Basilar membrane.	Tube of the cochlea.	Major axis of oval window.	Second whorl.
Number of whorls	·780 ± ·040 (− ·042 ± ·110)*	·060 ± ·093	·051 ± ·094	·000 ± ·093
Second whorl	·494 ± ·077	·500 ± ·070	·094 ± ·093	
Major axis of oval window	− ·069 ± ·102	·362 ± ·082		
Diameter of tube of cochlea	·325 ± ·101			

* Between the number of whorls and the index of the basilar membrane contained in the first two whorls.

10. There is a high correlation between the number of whorls and the index of the basilar membrane, but not between the former and any other cochlear index. This confirms the conclusion of (5) that variation in the number of whorls from the typical number two and a half occurs without any alteration in the basal plan of the cochlea; extra turns are added on at the top of the cochlea by simple continuation of the whorl, or if the turns are fewer than usual, the whorl simply stops short at the required point. The cochlea does not grow by accretions at the base as a shell does, but by accretions at the apex; if it grew in the former way, all the dimensions of the cochlea would be correlated highly and positively with the number of its whorls, which is not the case; there is not even any correlation between the number of turns and the index of the length of the basilar membrane contained in the first two whorls (*vide* note to Table III).

11. There is also a positive correlation—practically certain, if not quite decided—between the index of the basilar membrane and the index of the second whorl. This evidently means that the basilar membrane may be increased by an increase in the diameter of the second whorl as well as by

the addition of further whorls to the number that was more remotely inherited.

This result may seem to contradict that reached above (6), that there is a fair sign of a negative correlation between the absolute length of the basilar membrane or the diameter of the lowest whorl and the index of the second whorl (diameter of the second whorl divided by that of the lowest whorl). But there is really no contradiction. For there is no correlation between the absolute length of the basilar membrane and the index of the basilar membrane. Therefore there may well be an increased rate of curvature for absolutely long basilar membranes (large cochleas) and a decreased rate for relatively long ones.

12. When the index of the second whorl varies, the index of the diameter of the tube of the cochlea varies in the same direction. This is a practically certain and almost decided correlation. We might well expect a cochlear tube that is thicker than usual to coil less willingly, as it were, and so to give a wide second whorl. If the other dimensions of the cochlea, including the number of whorls, then remained typical, the basilar membrane would be longer for that organ than it usually is for any organ.

13. The correlation between the index of the basilar membrane and that of the diameter of the cochlear tube at its base is low and uncertain, being only three times the probable error. This would confirm the inferences made in (11) and (12) that in these connections the diameter of the cochlear tube, not the basilar membrane, is the leading variant.

14. This is further supported by the correlation indicated between the indexes of the major axis of the oval window and of the cochlear tube. There is no correlation between the former and the index of the basilar membrane.

We may sum up again by saying that there are two sources of change in the length of the basilar membrane. The chief one is its own absolute increase in length, which appears in a greater number of whorls than usual. The other is the relative increase in the diameter of the tube of the cochlea. There are no other internal variations in the dimensions of the cochlea than these.

15. In Table IV will be found the ranking of the mammals according to the index of the basilar membrane. The column marked "No." gives the order of succession in which the organs measured are given in Dr. Gray's two volumes, but Dr. Gray himself numbered only the somewhat smaller number of photographs in his books. The absolute length of the basilar membrane is my estimate from Gray's photographs. For the other measurements upon which this paper is based, see Gray's two volumes.

Table IV.

No.	Animal.	Index of basilar membrane.	No. of whorls.	Absolute length of basilar membrane.	Ranking of basilar membrane.
				mm.	
20	Common Weasel	8·16	3½	20·4	10
18	Mongoose	7·86	3	27·5	17
39	Capybara	7·34	4¼	51·4	37
37	Hairy-footed Jerboa	7·08	2¼	17·7	7
47	Vulpine Phalanger	6·92	3¼	27·7	18
12	Tiger	6·55	3¼	52·4	40
2	Yellow-faced Baboon	6·47	3½	38·8	31
29	Common Pig	6·40	3½	35·2	25
5	Hoolheur Monkey	6·37	2¾	36·6	27
19	Otter	6·35	3	25·4	13
17	Aard Wolf	6·34	3¼	44·4	35
16	Dog	6·16	3¼	38·5	30
3	Black Ape	6·12	2¾	36·7	28
31	Three-toed Sloth	5·93	2¼	32·6	24
13	Lion	5·82	3¼	52·4	27
35	Common Rat	5·80	2¼	14·5	4
7	Common Marmoset	5·69	2¾	25·6	14
4	Green Monkey	5·66	2¾	29·7	20
38	Common Squirrel	5·60	2¾	18·2	8
9	Slow Loris	5·52	2¼	20·7	11
25	Beisa Antelope	5·47	2½	41·0	32
51	Brush-tailed Phascologale	5·43	2¼	16·3	5
52	Short-nosed Bandicoot	5·43	2¼	16·3	5
45	Brush-tailed Wallaby	5·38	3	24·2	12
10	Indian Fruit Bat	5·34	2	18·7	9
22	Common Seal	5·31	2¼	53·1	41
8	Mongoose Lemur	5·28	2¼	26·4	16
49	Pouched Jerboa Mouse	5·25	1¾	10·5	2
21	Crab-eating Racoon	5·25	2¼	31·5	23
26	Indian Gazelle	5·24	2¼	36·7	28
46	Black-faced Kangaroo	5·22	2¾	31·3	22
30	Horse	5·18	2¼	46·6	36
23	Grey Seal	5·18	2	51·8	39
42	Whale	5·11	2	104·7	42
1	Man	5·10	2¾	42·1	34
28	Dromedary	4·90	2¾	51·4	37
32	Tamanduan Anteater	4·89	2	25·7	15
41	Common Mole	4·65	1¾	9·3	1
24	Cape Sea Lion	4·65	2¼	27·9	19
27	Common Sheep	4·26	2¼	29·8	21
43	Porpoise	4·21	1¾	35·8	26
40	Hedgehog	3·97	1¼	11·9	3
44	Sea Cow	3·95	1¼	41·5	33

This ranking can hardly be taken strictly as it stands, but it will be valid on broader lines. Half the number of organs measured have an index of five and a fraction. An index of over 6·5 may safely be held to be unusually large, an index of below 4·5 unusually small. I should divide the whole group into two parts at the index of 5·5; that gives two rather characteristic groups, in the first of which are many carnivora and some

much hunted animals, while the lower group consists mainly of herbivorous, insectivorous, and aquatic creatures.

It is of interest to note how low in the scale man stands. He is associated with the horse, the whale, the dromedary, the mole, the sheep, the hedgehog, and the sea-cow. Whatever an increased basilar membrane means functionally for hearing, whether it be a greater sensitivity or a greater pitch-range of hearing, or both, man can hardly claim to have any high power therein, if we may judge from the general impression of the power given by his associates in this Table. Amongst Dr. Gray's photographs there are depicted many more regular and beautiful organs than man's. Music may require a great mind and a great soul, but it can hardly presuppose a very fine receptor organ.

[16. In Table IV the positive correlation between the index of the basilar membrane and the number of whorls (*cf.* Table III) may be seen at a glance. But minor deviations from it are also apparent; for example, one might have expected the capybara with 4¼ turns to have the highest basilar index. And it is striking that the next organ on the list has only 2½ turns. Obviously some other variant must have caused these "short-time oscillations," so to speak. The measurements given for the capybara's organ suggest a likely cause. The photograph of that organ shows a high rate of curvature, the relation of the diameter of the second whorl to that of the first being only 64 per cent. So I reckoned a short-time fluctuation in relation to rate of curvature with the help of moving averages (groups of five organs) and the modifications of Karl Pearson's formula used in connection therewith, and found it to be $-0{\cdot}474 \pm 0{\cdot}083$. That is very nearly a decided correlation. And it indicates that an increased rate of curvature calls for more whorls, a wider second whorl for fewer whorls, than would generally be required by the functional demands summed together in the index of the basilar membrane.

This result might have been deduced from the results stated in Part A. For if extra whorls are added on at the apex without alteration of the other dimensions, the extra amount of basilar membrane required in a cochlea with a wide second whorl will go into fewer turns than in a cochlea with a narrow second whorl. But it is well to derive it independently, for it clarifies the functional significance of the basilar index, showing it to be independent of the other variable features, and helps towards a reasoned understanding of the architectonic of the cochlea.—*Added December*, 1916.]

17. Table V gives the ranking for the birds, if an index is taken from the highest correlation—the length of the tube of the cochlea divided by the major axis of the oval window. Here, again, a carnivore heads the list,

followed appropriately by the song thrush. By this method of calculation the ducked-billed platypus, which has a cochlea of approximately avian type, shows an index of only 2·0—the lowest in the Table.

Table V.

No.	Animal.	Index of basilar membrane.
63	Sparrow Hawk	4·0
74	Song Thrush	3·5
71	Slender-billed Cockatoo	3·3
68	Crowned Crane	3·0
70	Crowned Pigeon	3·0
72	Burrowing Owl	3·0
58	Cape Gannet	3·0
64	Great Tinamou	2·75
60	Night Heron	2·66
59	Cormorant	2·57
57	Red-throated Diver	2·57
56	Apteryx	2·5
54	Ostrich	2·4
66	Red Grouse	2·33
67	Porphyris	2·28
62	Buzzard	2·25
73	Carrion Crow	2·0

C. *General Remarks.*—How do these results modify the generally accepted notions regarding the mode of action of the cochlea? The chief point is that they clear up our ideas. The cochlea, it may be said, has usually been vaguely apprehended as a curious organ, and, like the sensations it yields, rather unique in its nature. But just as a renewed psychological analysis of the sensations of sound brings them into clear accord with the structure of the sensations of the other senses,* so this study brings the cochlea morphologically into line with the eye. Each is an organ in which certain main relationships hold universally, with minor variations for special purposes. The cochlea is perhaps more dependent upon the gross bulk of the organism than is the eye.

Little is yet known directly about the auditory functions of the different animals. But the series given under "Animal" in Table IV seems more acceptable as an indication of auditory power than that formed by increase in the absolute length of the basilar membrane. We should hardly expect the finest hearing in the whale, the seal, (the tiger), the grey seal, the dromedary, (the capybara), the horse, (the aard-wolf, man?), and the sea-cow—which is the order of decreasing cochlear dimensions. And the close

* *Cf.* my 'Psychology of Sound,' Cambridge, 1917.

correlation of cochlear sizes with gross bulk makes any such assumption highly untenable. If this be granted, then it would seem that the absolute length of the basilar membrane (and, therewith, the scale of dimensions of the cochlea as a whole) is neither relatively, nor perhaps at all, important for efficiency of hearing. It is rather the index of the basilar membrane that counts, its length relatively to the size of the organ as a whole. This conclusion is supported by the fact elucidated above (10) that the chief internal variation of cochlear structure is the number of whorls or the relative length of the basilar membrane.

But if we thus abrogate the importance of the absolute length of the basilar membrane, may we not also sacrifice the absolute breadth of it as well, and within limits the absolute number of its transverse fibres? These limits seem inevitable, in so far as pitch discrimination could hardly be refined much beyond the subdivision given by the transverse fibres; but in a larger organ it need not approach even within some distance of that subdivision. I have elsewhere (*op. cit.*) tried to prove that the most important property of the basilar membrane in its longitudinal aspect is its elasticity; and that a functional or psychical hearing, in all respects similar or parallel to our own, in so far as its sensory integrations are concerned, might be got from any absolute length of basilar membrane. The perfection of its efficiency would depend only upon the elasticity of the membrane and its relative length. The results of this paper bear well with this theory. On the other hand, it must be noted that these results are not directly incompatible with the hypotheses of any of the chief physiological theories of hearing.

The Rôle *of the Phagocyte in Cerebro-spinal Meningitis.*

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[Plates 13 and 14.]

The more or less constant presence of the meningococcus in the spinal fluid of cases of cerebro-spinal fever, has led to many suggestions as to how this microbe gains admission to the spinal canal. It has been assumed that either there is a direct passage of the organism from the naso-pharynx to the cerebral meninges, or that transmission takes place through the blood or lymph channels.

The membranes which enclose the spinal fluid, however, present a serious obstacle to the passage of the microbe, and in fact it is doubtful if, in the living tissues, such a passage could take place.*

If passage by means of the blood stream or the lymph were easy, on the other hand, we might expect to find meningitis a frequent complication of streptococcal septicæmia—which is not the case.†

Though the spinal meninges are probably impervious to the passage of free meningococci, they certainly do not prevent the passage of leucocytes.

Normal healthy spinal fluid contains a few wandering cells, mostly of the lymphocyte variety. In cerebro-spinal fever the fluid is invariably crowded with large numbers of polymorph leucocytes, which then frequently contain many meningococci. In such cases we have often noticed that the microbes within the leucocytes have undergone little change, and show no obvious signs of degeneration or digestion. In staining reaction, moreover, they show no appreciable difference from those lying without the phagocytes.

* To obviate this difficulty it has been suggested that the meningococcus starts life as a filterable virus, and that in this form it successfully enters the canal. In none of our cases (with a Doulton filter) have we been able to obtain a filtrate that would give any growth when planted out on chocolate medium.

† No attempt has been made in the present paper to consider the question of the infection of the meninges by way of the lymph channels. If this is brought about by the flow of lymph from the nasal spaces through those surrounding the olfactory nerves to the subdural and subarachnoid spaces of the cranium, then we have to assume a peculiar susceptibility to infection on the part of the membranes surrounding these spaces, by the meningococcus, or otherwise we should find meningitis a frequent complication of the usual infections of the nose.

Is it possible that, under certain conditions, the meningococcus can remain alive within the leucocytes? If such should prove to be the case, then wandering leucocytes might convey living meningococci into the spinal canal and thus bring about infection.

More than a quarter of a century ago, Ruffer* drew attention to the fact that, in sections of the rabbit's tonsil, the leucocytes are frequently seen crowded with bacteria, which seem to have undergone little digestion. To all appearances they seem to resemble free germs.

Since then many similar observations have been made, notably by Metchnikoff,† and his pupils, Adami,‡ Nicholls,§ Ford,‖ and many others.

In 1895, Bordet¶ found that cholera spirilla, injected into the blood stream of cholera-immune animals, are taken up by the leucocytes before they are subjected to lysis by the circulating antibodies.

Metchnikoff,† Levaditi,** and Briscoe†† similarly have shown that red blood cells injected into previously immunised animals may be taken up by the leucocytes before they can be hæmolysed.

Rous and Jones‡‡ have shown that leucocytes can protect typhoid bacilli, after ingestion, from the toxic action of a N/150 KCN solution. This strength of KCN they showed was highly lethal to the unprotected germs.§§ They also proved that living leucocytes can shield these bacilli from the action of a strong homologous serum, as the serum is unable to exert any action on those germs within the leucocytes. They were able subsequently to recover living germs from these leucocytes.

McKee‖‖ has found that in ophthalmia neonatorum (gonococcal), the cells of the conjunctival epithelium can take up the gonococcus in large numbers, and that these cocci are not killed by the silver nitrate when the eye is subsequently washed out with this solution. Similar observations have been made on the urethral epithelium by other investigators. We have thus a clear explanation of the cause or source of recurrent infection, so frequent where this germ is concerned.

* Ruffer, 'Brit. Med. Journ.,' 1890.

† 'Metchnikoff, 'L'Immunité dans les Maladies Infectieuses,' Paris.

‡ Adami, Abbot, and Nicholson, 'Journ. Exp. Med.,' vol. 4 (1899).

§ Nicholls, 'Journ. Med. Resch.,' N.S., vol. 6 (1904).

‖ Ford, 'Journ. Hyg.,' vol. 1 (1901).

¶ Bordet, 'Ann. de l'Inst. Past.,' vol. 9 (1895).

** Levaditi, 'Ann. de l'Inst. Past.,' vol. 16 (1902).

†† Briscoe, 'Journ. Path. and Bact.,' vol. 12 (1908).

‡‡ Rous and Jones, 'Journ. Exp. Med.,' vol. 23 (1916).

§§ We did not find a N/150 KCN solution made up in Lock's solution toxic for the meningococcus after 3 hours' exposure to its action at 37° C.

‖‖ McKee, 'Ophthalmic Record,' Chicago, January, 1912.

It has been recently pointed out by several observers that it is doubtful if the polymorph leucocytes of the body are capable of successfully attacking and digesting bacteria of the acid-fast group. It has been shown by Tschernortusky* that extracts of these leucocytes, although containing the usual proteolytic enzymes, are remarkably deficient in lipase. They are probably unable for this reason to digest the waxy substance forming the characteristic feature in the organisation of this group of bacteria.

This is borne out by the work of Terry,* in Zinsser's laboratory, which shows that rat leprosy bacilli may be kept within the leucocytes for weeks without undergoing any apparent change or losing their acid-fast properties; whereas the same bacilli, as shown by Zinsser and Cary,† are rapidly digested when taken up by the spleen tissue cells grown in blood plasma.

In the light of the foregoing observations, it seemed to us that it should be possible to demonstrate experimentally the viability or non-viability of the meningococcus within the leucocytes in cerebro-spinal fever. The following experiments have been attempted with this object. The recovery of living cocci from the leucocytes of undoubted cases of cerebro-spinal fever ought conclusively to establish this point.

Our first object is to show that we are using a medium which is quite favourable to the growth of the meningococcus. Our experiments will give erroneous results, if, for example, a medium is used on which the organism will only grow when implanted in massive amount, for under such conditions we are not certain that the germ is dead, although it fails to grow. The tests should carry conviction on this point.

The medium used in our experiments is that described by one of us.‡ This is made from defibrinated bullock's blood and glucose, with trypsin agar as a base. To avoid the cumbrous title "blood-agar-glucose," it will be referred to briefly as "chocolate medium," from the similarity of its appearance to this substance.

To determine its power of growing the meningococcus, it was compared with a number of media. The results of this comparison will appear in detail elsewhere. Briefly, the conclusion is reached that if a 24-hour culture on chocolate medium is emulsified in distilled water and diluted down and planted out, then, presumably through a certain proportion of these germs being dead, it is found that a certain minimum number of organisms are necessary to the implantation for growth to take place. This minimum varies with the different media in accordance with their capacity for

* Quoted from Zinsser, 'Infection and Resistance,' p. 284 (1914).

† Zinsser and Cary, 'Journ. Amer. Med. Ass.' (1912).

‡ Crowe, 'Lancet,' November 21, 1915.

growing the meningococcus. It varies also with the character of the strain of meningococcus employed, as some grow very rapidly on artificial media, while others grow slowly.

The following Table gives roughly the number of germs of Strain "P," which, having been planted out in similar definite quantities on the following media, gave growth after incubation at 37° C., for 48 hours:—

Name of medium.	Growth positive.	Growth negative.
War Office legumen tryp-agar	50,000,000 germs	10,000,000 germs.
Buchanan's medium	—	100,000,000 ,,
Ascitic legumen agar	10,000,000 germs	1,000,000 ,,
Amino-acid agar (Cole's)	—	100,000,000 ,,
Amino-acid agar, mixed with fresh blood	10,000,000 germs	1,000,000 ,,
Blood agar	10,000,000 ,,	1,000,000 ,,
Blood smeared agar	10,000,000 ,,	1,000,000 ,,
Egg medium	—	100,000,000 ,,
Chocolate medium	1,000 ,,	—

In the above Table a thousand germs was the smallest number planted out. Chocolate medium will, however, readily grow the meningococcus in inoculations of 500 cocci. In implantations made from glucose broth cultures, this number is reduced still lower, as presumably, in this instance, all the cocci are alive. Experiment has shown that growth will then take place, on this medium, in implantations of as few as 20 or 30 organisms.

We drew the conclusion from the above facts (1) that chocolate medium was well adapted to our requirements, and was suitable for testing the viability of the meningococcus, as far as this can be determined on artificial media; (2) that, given a reasonable number of germs to an implantation, failure to grow on our medium denoted the presence of almost a negligible number of cocci.

One of us has shown that nearly all strains of the meningococcus succumb rapidly in the presence of 0·85 per cent. NaCl solution, on exposure to its action for a short while.* All the strains of the meningococcus used in the following experiments were found to be highly susceptible to the toxic action of dilute NaCl. While a 0·85 per cent. NaCl solution is toxic, a 1·5 per cent. NaCl solution is more or less harmless. In the following experiments, taking fresh spinal fluid obtained from lumbar puncture of cerebro-spinal fever patients, and lightly centrifuging down the leucocytes, we have made use of this toxic action of a 0·85 per cent. NaCl solution to kill all the meningococci outside or attached to the leucocytes, while those enclosed within them are protected from its action.

* Shearer, "On the Toxic Action of Dilute Pure Sodium Chloride Solutions on the Meningococcus," 'Roy. Soc. Proc.,' B, vol. 89, p. 440 (1916).

Experiment 1.

Expt. 1.—Fresh "Sloan" spinal fluid, rich in meningococci, both within and without the leucocytes, was centrifuged lightly to bring down the leucocytes.

Treatment.	Growth, 24 hours.
(1) Leucocytic deposit was washed in 1 per cent. sterile glucose, centrifuged three times and planted out	Good.
(2) Leucocytic deposit was washed in 1 per cent. sterile glucose, centrifuged six times and planted out	Good.
(3) Upper portion of original centrifuged spinal fluid was centrifuged hard for 15 minutes and planted out	Good. Far the best.

In (1) fresh leucocytic deposit containing many meningococci within the leucocytes, was washed three times in 1 per cent. sterile glucose and centrifuged and planted out on a plate of chocolate medium. The glucose exerts no harmful action on the cocci, while it disorganises and probably kills the leucocytes through its hypertonic action. The washing has removed practically all the free cocci. At the end of 24 hours there was a good growth on the plate. This growth could hardly be accounted for by supposing it to be derived from the few free cocci that may have remained over from the washings.

To test this further, in (2) the deposit was washed six instead of three times and planted out in a similar fashion. The result was the same as in (1). Here the extra washing had no effect in lessening the amount of the growth, which was as great as in (1).

In (3) the upper part of the original centrifuged spinal fluid, containing few leucocytes but numbers of free cocci, was centrifuged hard for 15 minutes. On planting this out, as was to be expected, growth was immediate and greater than in (1) and (2).

The conclusion to be drawn from this experiment is open to question, as it cannot be said with any certainty that all the loose germs in (1) and (2) were removed by the repeated glucose washings.

Moreover, the glucose itself is a stimulant to the growth of the meningococcus. The following experiment brings out this point, but at the same time shows that this effect is not very great, and probably is not sufficient to disturb the result of our experiments.

A fairly thin emulsion of the meningococcus used in Experiment 1 (500 millions to the cubic centimetre) was made up in distilled water. Successive dilutions were made by mixing 5 c.mm. of this emulsion with 25 c.mm. of 1 per cent. sterile glucose broth, removing 5 c.mm. and mixing with a second 25 c.mm. of broth, and so on through eight dilutions. A sterile

camel's-hair brush was dipped in the highest dilution, and drawn across the surface of the plate. The remaining seven dilutions were treated in a similar manner. In the end there were eight parallel lines, taking up one half of the plate, each representing a dilution of the meningococcus in glucose. The other portion of the plate was treated in a similar manner with eight successive dilutions of the same quantity of emulsion, in distilled water. The plate was then incubated at 37° C. for 48 hours. A photograph of the plate is shown in fig. 3, after 24 hours' growth. The action of the glucose in accelerating growth will be seen from an examination of the successive lines on the one, as compared with the other half of the plate. A close comparison of the two top lines with the two bottom lines of growth brings out the action of the glucose. In this instance it is slight and inconsiderable. The action of the glucose, therefore, may be safely neglected as a source of grave error.

There is a further point to be considered with regard to this and all the following experiments. Are we certain that the leucocytes we are using in these experiments are alive and not dead?

We endeavoured to settle this point by two tests.

Firstly. By an examination of fresh leucocytic deposit of spinal fluid on the warm stage. This elucidated the fact that if the spinal fluid was freshly drawn by lumbar puncture from a cerebro-spinal fever case and was only cloudy and turbid in appearance, and not at all purulent, then practically all the leucocytes were alive, as they always showed vigorous amœboid movement when placed on the warm stage. If the fluid was purulent, then most of the leucocytes were dead.

Secondly. It has been shown by Rous and Jones* that a dilute solution of trypan blue in Ringer's solution readily stains the nuclei of dead leucocytes, while it will not touch those of the living cells. We have confirmed this point for human leucocytes found in the spinal fluid of cerebro-spinal fever cases. To this end, freshly drawn fluid containing leucocytes was taken which showed obvious amœboid movement on the warm stage. This was divided into two portions. The leucocytes of one portion were stained for a few minutes in dilute trypan blue in Ringer's solution, and then examined under the microscope; none of them took the stain. To the second portion a little alcohol was added to kill them, and they were then stained as before; all immediately took up the stain.

We then applied the trypan blue method of staining to a number of leucocytic deposits obtained from cerebro-spinal fever cases, such as those used in

* Rous and Jones, 'Journ. Exp. Med.,' vol. 23 (1916).

the following experiments. The trypan blue test bore out the results obtained with the warm stage. The leucocytes do not take up the stain as long as the spinal fluid is simply cloudy and turbid and no traces of pus present. This test, moreover, would seem to show that human leucocytes can remain alive for 2–3 days in the spinal fluid, when this is allowed to stand at room temperature under sterile conditions.

In the following experiments we made use of spinal fluid which we had every reason to believe, in view of the above tests, contained living leucocytes. In no instance did we use fluid showing the presence of pus cells. The results of the experiments themselves preclude, moreover, the possibility that the majority of the leucocytes were dead.

Experiment 2.

In this, as in the former experiment, leucocytic deposit of fresh "Sloan" spinal fluid, containing this time practically no free germs, but many within the leucocytes, was removed and washed. Part only was washed in 1 per cent. glucose. The remainder was washed in 0·85 per cent. NaCl. The washings in both instances were repeated 16 times, to make certain, as far as possible, that no free germs should remain.

Microscopic examination of the deposit after washing in glucose showed a slight disorganisation of the leucocytes, while those washed in saline showed no change. The normal saline, while not affecting the leucocytes, presumably exerted a toxic action on the free germs or those attached to the exterior surface of the leucocytes. Those within were protected from its action.

Expt. 2.—Fresh "Sloan" spinal fluid, containing no free cocci, but large numbers within the leucocytes, was centrifuged lightly to bring down leucocytes.

Treatment.	Growth, 24 hours.
(1) Leucocytic deposit of spinal fluid washed 16 times in 1 per cent. sterile glucose, and planted out	Good.
(2) Leucocytic deposit of spinal fluid washed 16 times in sterile 0·85 per cent. NaCl, and planted out	Good, but delayed: at end of 48 hours, same as (1).

In this experiment (1) we got a rapid and immediate growth covering nearly the whole of the plate at the end of 24 hours. In (2) the growth only attains to the amount of that of (1) at the end of 48 hours. In both (1) and (2) practically all free cocci have been eliminated by the repeated washings. Those remaining over or attached to the surface of the leucocytes in (2) have been killed or injured by the toxic effect of the saline. Those

attached to the surface of the leucocytes in (1) have been uninjured by the glucose. The immediate growth of (1), as compared with (2), is probably to be sought for in the fact that in (1) the glucose disorganises and destroys the leucocytes, so that the microbes they contain are more rapidly set free to grow, while in (2) the normal saline keeps the leucocytes longer intact, and their microbes are only set free to grow after a considerable interval.

Experiment 3.

This experiment was a repetition of 2, with spinal fluid from a case whose condition was critical when the lumbar puncture was made. The leucocytes were probably less favourable for the experiment than in the former instance. Practically the same result, however, was obtained. In figs. 1 and 2 are shown photographs of the growths of the glucose and saline deposits of the experiment respectively at the end of 24 hours. It will be seen that the glucose-washed deposit has grown much more than that in the normal saline. At the end of 48 hours the growth on the two plates was practically equal.

How far are we justified in drawing conclusions from Experiments 2 and 3? It is hard to believe that the abundant growths in both experiments with the glucose-washed deposit is due entirely to the accelerating action of the glucose on the few microbes remaining attached to the exterior of the leucocytes. That is, if we suppose all the microbes within the leucocytes to be dead. Taking into consideration all the facts of the case, we think there is a certain amount of evidence in favour of the view that the microbes were alive within the leucocytes.

Experiment 4.

Leucocytic deposit from the fresh spinal fluid (N. H.) was washed three times with sterile 0·85 per cent. saline and divided into two portions. One was kept intact, while the other was crushed with sterile glass powder. Samples of each were planted out, and the remainder were left to stand for three hours in a large bulk of normal saline. They were then thoroughly centrifuged and planted out.

Expt. 4.—Leucocytic deposit from spinal fluid (N. H.), containing many free and enclosed cocci, was washed three times in 0·85 per cent. NaCl, and divided into two portions. Portion (*a*) was kept intact, while portion (*b*) was crushed up with sterile glass powder. Samples of each were then planted out, and the remaining portions were then allowed to stand in sterile 0·85 per cent. NaCl for three hours. They were then centrifuged thoroughly and planted out.

Treatment.	Growth, 24 hours.
No saline—	
(*a*) Leucocytic deposit planted out immediately	Good.
(*b*) Leucocytic deposit crushed and planted immediately	Good; better than (*a*).
Saline for three hours—	
(*a*1) Leucocytic deposit planted out after three hours standing in 0·85 per cent. NaCl	Delayed and poor.
(*b*1) Leucocytic deposit crushed and planted out after three hours standing in 0·85 per cent. NaCl	One colony only.

At this point it seemed to us that it ought to be possible by making use of the opsonic technique to arrange an experiment which might provide a crucial test of our hypothesis.

The meningococcus, when first isolated from the spinal canal, is not susceptible of being taken up by the phagocytes in the presence of normal serum. There are said to be exceptions to this rule (Kolle and Wassermann), but so far in our work we have met none. During sub-culture the organism gradually loses the power of antagonising the action of the leucocytes, until after a month or six weeks of growth under laboratory conditions, a very considerable opsonic effect can be demonstrated. Immune serum on the other hand is a most powerful "opsoniser" of freshly isolated cocci. The contrast between an opsonic film prepared with normal serum and that prepared with immune is very striking.

Since it was our desire to obtain leucocytes filled with meningococci, clearly we were right to employ freshly isolated cultures, and sensitise the emulsion made from such a culture with the serum of the patient from whom the germ was isolated. As a control we desired to utilise leucocytes with very few germs inside them; the same emulsion sensitised by a non-immune normal serum gave us what we wished.

Remembering the poisonous action of the normal saline when applied to meningococci, we could readily free the leucocytes which we had charged or intentionally left uncharged from any loose germs which might not have been ingested, and in this way our results were not obscured by the growth of the organisms untouched by the phagocytes. We had to be careful, however, not to emulsify the culture used for the purpose of making the opsonic mixture in normal saline, since the toxic action of the saline would have come into play and seriously interfered with the result of the experiment. It would have been very difficult under these circumstances to appraise properly the leucocytic content, as many of the germs would have been killed by the saline beforehand.

Emulsion made up in 1·5 per cent. saline, however, completely avoids this difficulty, as this strength of saline has little or no deleterious action on the meningococcus and does not interfere with the leucocytes.

Having thus obtained leucocytes ready charged with germs, and also for control purposes other leucocytes almost free from germs, it only remained to destroy a certain proportion of the former by some means or other. To this end we again employed the method of grinding with sterile powdered glass. Finally, by submitting both crushed and uncrushed cells and also the control leucocytes to the action of normal saline, we were able to determine whether or no the organism within the intact leucocytes were still viable. Those which had been freed by the destruction of the leucocytes should have been killed by the unrestrained action of the normal saline.

For the sake of greater clarity, it is perhaps as well briefly to tabulate the conditions on which this crucial experiment depends.

They are as follows :—

1. An immune serum exerts a powerful action in stimulating the leucocytes to take up the germs. We can, therefore, fill leucocytes at will with meningococci.

2. Since freshly isolated meningococci are but slightly susceptible of being attacked by the phagocytes with normal serum, as a control, an opsonic mixture where normal serum is substituted for immune will provide us with leucocytes fairly free of meningococci.

3. We know from the foregoing experiments that normal saline will kill all meningococci except those ingested and protected by the leucocytes.

We can finally destroy by mechanical means some of the leucocytes containing meningococci, and resubmit both these and intact leucocytes to the action of normal saline. If our hypothesis that leucocytes protect meningococci from death is correct, then from the intact leucocytes growth will take place.

Provided also that the normal saline is poisonous to the germ, growth from the control leucocytes (treated with normal saline) will only be slight.

Experiment 5.

Opsonic mixtures $\begin{cases} \text{S} = \text{Coccus S} + \text{washed leucocytes and serum S (immune).} \\ \text{P} = \text{Coccus S} + \text{washed leucocytes and normal serum P.} \end{cases}$

Coccus S is a recently isolated coccus from patient S. suffering from cerebro-spinal fever. Serum of this patient and a normal man P. was drawn the day before the experiment. A 24-hour culture of coccus S was emulsified in 1·5 per cent. saline to prevent lysis, and the mixtures put up in equal parts of emulsion, serum and leucocytes, and incubated for

five minutes. A sample was spread on a film and the remainder of each specimen mixed with the saline and washed three times. A further sample was planted out 40 minutes after the mixtures were put up. After two hours half the centrifuged deposit of mixture S in saline was crushed with sterile glass powder. One and a half hours later, samples were again planted, and this procedure was repeated at intervals of 6 and 24 hours after the commencement of the experiment.

Microscopic appearance of the mixtures:—

(1) "S" mixture showed extreme agglutination, nearly all the leucocytes crowded with cocci.

(2) "P" mixture (control), no agglutination, only a few cocci in the near neighbourhood of the cells. It was doubtful if any microbes at all had been taken up by the leucocytes.

Experiment 5. (See figs. 6 and 7.)

Time.	S.*		P.*
I. 40 minutes in saline ...	Area planted, quite covered.		Very few colonies.
II. 3 hours in saline	Uncrushed. Area planted out quite covered	Crushed (Cr.). Area planted out quite covered	Very few colonies.
III. 6 hours in saline	Covered	No growth	Growth discrete.
IV. 24 hours in saline	Covered	No growth	Covered.

* S = Coccus S + washed leucocytes and serum S (immune).
* P = Coccus S + washed leucocytes and normal serum P.

In figs. 6 and 7 are shown photographs of the result of this experiment after 24 and 48 hours' growth, respectively.

Row I, P.—Very few colonies growing owing to the loose germs being washed away, whereas in S the leucocytes hold the organisms.

Row II.—The excellent growth in the crushed area (Cr.) shows that the manipulation with the glass powder did not destroy the vitality of the germs, although no leucocytes were left intact (confirmed microscopically).

Row III.—In column (Cr.) where crushed leucocytic deposit is planted, the free germs have succumbed to the action of the saline. There is no growth. On the left hand, however, where the leucocytes were intact, growth was maximal.

Row III and IV, P.—Growth steadily increasing. We must suppose that a few germs have been taken up by the leucocytes. These have probably

increased within their hosts; hence the progressive increase in the amount of growth. It is also possible that this growth is due to the blood being allowed to stand for such a length of time at incubator temperature. It was noticed that at the end of 24 hours' time it had undergone considerable hæmolysis. This would liberate an appreciable quantity of calcium salts which would in turn destroy the action of the saline. Thus a condition would be brought about which would be favourable to an excessive growth of the few germs that might have been taken up by the leucocytes. The accessory growth factor known to be present in the blood would also come into play, and, the inhibiting toxic action of the normal saline being abolished, rapid growth would take place.*

The main conclusion of our former experiments is therefore borne out again in Experiment 5. Taking Row III of this experiment, the large growth in the first area, where the leucocytes have remained whole, although exposed to the toxic action of the saline for six hours, as compared with the complete absence of any growth in the second area, where they have been crushed with glass powder and have come under the direct action of the saline, is very striking and is beyond dispute.

Experiment 6.

Emulsions of two strains were submitted to the action of the leucocytes. Strain W was an old laboratory culture, isolated from the spinal canal nine months previously. Strain S 1 had been isolated less than a fortnight.

Opsonic mixtures	A.—Coccus	W +	leucocytes	+	normal serum.
	B.— „	S1 +	„	+	„ „
	C.— „	W +	„	+	immune serum.
	D.— „	S1 +	„	+	„ „

Microscopic appearance of the mixtures after 15 minutes' incubation :—

"A."—Showed no agglutination, but the leucocytes contained many cocci, many shades of cocci, and the staining of nearly all of them within the cells was poor, suggesting partial digestion.

"B."—Showed no agglutination, and very little ingestion of the germs. Organisms stained well.

* It has long been remarked that all blood media grow the meningococcus with great readiness; similar results can be obtained with watery and alcoholic extracts of blood. One of our number (C. S.) hopes to show in a forthcoming paper that in nasal secretion such a body is also present, which is undoubtedly of the nature of an accessory food body in all its properties. It is possible that this body present in blood is similar to that found in the nasal secretion, the nasal secretion, in short, obtaining it from the blood.

"C."—Showed an intense degree of ingestion, and nearly all the free organisms aggregated into clumps.

"D."—Showed extreme agglutination and some degree of ingestion, but not nearly so much as in "C," a rather curious result, probably due to rapid agglutination preventing the full play of the leucocytes.

A portion of each mixture washed in 1·5 per cent. saline, and freed from leucocytes by the centrifuge, was planted out immediately, and in every instance growth took place. The remaining portions of the mixtures were shaken up with a large bulk of normal saline and incubated at 37° for two hours, then lightly centrifuged. The upper portion was afterwards drawn off and recentrifuged hard for a quarter of an hour, whilst the deposit was planted out. Finally, the deposit of the recentrifuged upper portion, which would contain free germs, was also planted out. The result is shown in the following Table.

Experiment 6.

Mixture.	Planted immediately to show viability of culture.	Planted after 2 hours in normal saline.	
		Leucocytic deposit.	Free germ deposit.
"A"	Good growth	No growth	No growth.
"B"	Good growth	One or two colonies	No growth.
"C"	Good growth	Good growth	Good growth.
"D"	Good growth	Good growth	Good growth.

At first sight this result was incomprehensible, since it appeared that the immune serum of a patient (mixtures "C" and "D") had no bacteriological effect on his own germ or on a laboratory culture. Yet both were apparently killed by the serum of a normal man. But consideration of the foregoing experiments shows that in point of fact the result is quite consistent. The explanation would appear to be as follows:—

Mixture "A."—The old laboratory culture was taken up and killed by the leucocytes whilst free organisms succumbed to the normal saline.

Mixture "B."—This gave an identical result with Experiment 5 (*q.v.*). All loose germs were killed by the normal saline, whilst from the leucocytic deposit one or two colonies arose from odd germs, which had been ingested and protected from the action of the saline by the leucocytes.

Mixture "C."—Here the dominating factor in the situation seems to be the agglutination, which, with an undiluted immune serum, may take place in less than a minute. Under the influence of this serum, aggregations of germs are taken up by the leucocytes, and, owing to the crowding of the

cells, fail to get digested. At the end of 15 minutes' incubation, the aggregations are very large, and the germs cannot be acted on by the normal saline. Living germs can, therefore, be demonstrated both within and without the leucocytes after two hours' incubation in normal saline.

In regard to mixture "D," the same explanation would apply.

Finally we would like to draw attention to the fact that in some cases of cerebro-spinal fever, the leucocytes of freshly drawn spinal fluid frequently show the meningococci growing out from them in large numbers. The germs can be seen filling the interior of the cells and actually bursting them open in places and growing forth in dense masses.

In one case under our care, "Hayes," this condition was very obvious. In fig. 8 is shown a microphotograph of some of the freshly drawn leucocytes of this case. In the centre of the figure is a large polymorph cell which has been burst open on one side by a dense mass of meningococci. A close examination of the fluid showed that almost every second cell was in a similar condition. Staining with trypan blue showed at the same time that relatively few of these cells were dead. It is clear that in instances like these the meningococci are plainly alive within the leucocytes.

Having now shown that the meningococcus can be alive within the leucocytes, the suggestion that the disease appears as the result of accidental carriage of the organism into the spinal canal by emigrating leucocytes assumes some credibility. Should this suggestion prove true, then in it we have an explanation of the fact that the disease is so seldom transmitted direct, but usually through the intervention of a "carrier." Ingestion of the germs by the phagocytes would have to be an essential factor in the propagation of the disease. As we have seen, the meningococcus when freshly isolated, is insusceptible of being ingested by the phagocytes in the presence of normal serum.

It is also true of the meningococcus when isolated from the naso-pharynx of a patient suffering from the disease in the early stage. In a recent case, where the organism was present in the naso-pharynx on the third day of the disease, a very thick emulsion of the germ was incubated with normal serum and washed leucocytes for a quarter of an hour at 37° C., and no trace of ingestion of the germ by the phagocytes could be observed.

At various times a considerable number of strains have been examined in this connection, and although they gave rather variable results in certain instances, on the whole we found that the further removed an organism is from the case in which it caused the disease, either in point of time or passage from throat to throat, the more susceptible does it become to

ingestion on the part of the phagocytes. At the same time it also becomes weaker, and may succumb to the lethal action of the serum or of the serum and the leucocytes combined. Thus the examination of the leucocytes when tested with an enfeebled throat strain shows them to be gorged with "shades" and poorly stained cocci. The substance of the leucocytes is vacuolated, suggesting the complete digestion of some of the germs taken up. This appearance is never seen in recently isolated spinal strains even when ingestion of the germs by the phagocytes is obtained with an immune serum.

If it were possible to trace a case of disease to a certain "carrier," eliminating any other possible source of infection, we ought to be able to demonstrate phagocytosis of the organism when isolated from the "carrier," although no ingestion on the part of the phagocytes would be observed in the same germ when isolated from the patient.

We were fortunate in coming across such a case, where the course of infection seemed to be beyond doubt. A patient developed cerebro-spinal fever after he had been in hospital a few days. None of his hospital contacts were "carriers" of the meningococcus. On investigating the camp from which he came we found one "carrier" with whom he had been in close contact in the same hut. In the presence of normal serum the germ from this man's throat was readily attacked by the phagocytes to a considerable degree. No ingestion on the part of the phagocytes could be demonstrated, however, after the passage of the germ through the patient. These strains, both spinal and nasal, we presume were the same, as they both behaved identically when tested with Gordon's monovalent serum Type 2. In this instance, then, it is hard to avoid believing that the man was infected from this "carrier," and it is interesting in the light of our experiments to note that the germ at the time of infection in this case was rapidly attacked by the phagocytes in the presence of normal serum.

Summary.

As the result of the foregoing experiments we think we have obtained good evidence for thinking that under certain conditions the meningococcus can be taken up by the leucocytes but not killed by them. In the case of freshly isolated strains we have seen that the leucocytes will not take them up at first. With old laboratory cultures, on the other hand, ingestion on the part of the phagocytes takes place with great rapidity. In a short time the germs are killed and completely digested by the leucocytes.

This happens also with the majority of the nasal strains we have examined from chronic "carriers" although they show great individual differences.

In the intermediate stage between the fresh spinal condition and the naso-pharyngeal state, it can be shown experimentally that they are taken up, but not killed, by the leucocytes. They can be recovered from them after a period of 24 or 48, or even 60 hours, and grown on artificial media. If we can believe they behave similarly within the body, then we can understand how they might be carried into the spinal canal and there set up infection.

It might also explain why direct infection (apart from the "carrier"), seldom, if ever, takes place in cerebro-spinal fever; that is from patient to patient, attendant, or physician, etc., the phagocytes refusing to take up the germs in their virulent condition. In the "carrier," on the other hand, in the majority of instances, the germs have lost their virulence so completely that they are taken up and immediately killed and digested.

In conclusion: if the method of infection is by leucocytic conveyance, then the reason why direct infection is so uncommon is clear. The virulent organism is unsusceptible of being attacked by the phagocytes. The longer the germs grow in the "carrier" throat, the more easily will they be ingested until a time is reached, when, on ingestion, they are also destroyed. Somewhere between these two extremes, infection may produce the disease. The organism is sufficiently weakened to give in to the leucocytic attack, but not to lose its life in the battle. Should infection occur at this point, the leucocytes will pick them up from the mucous membrane of the naso-pharynx, and in the course of their wanderings will sometimes carry them into the spinal canal. There the liberated organisms will set up the disease, at the same time re-acquiring the power of resisting the attacks of the leucocytes in the presence of normal serum.

Appendix.

The important part played by virulent non-ingestible strains of the meningococcus, as compared with virulent indigestible ones, in the light of the foregoing experiments, renders necessary some consideration of Rosenow's* remarkable results with virulent and non-virulent strains of the pneumococcus.

Rosenow has brought forward certain experiments to show: first, that a non-virulent pneumococcus strain does not absorb opsonin from a normal serum, and that it is always non-ingestible; secondly, if well washed it will become indigestible. He considers these properties to be brought about through the possession on the part of the virulent cocci, of a specific

* Rosenow, 'Journ. Inf. Dis.,' vol. 4 (1907).

substance which he calls "virulin"—presumably this attaches itself to the microbes as a covering, as it is removed by washing; thirdly, a non-virulent strain of the pneumococcus may be rendered virulent by incubating it a certain time in a saline extract of a virulent one. When thus treated it was no longer indigestible, and would kill a guinea-pig, which he proved it would not do previous to this treatment.

His experiments, however, do not carry conviction. The loss of opsonic effect which he describes might be explained by the fact that the extract in which the avirulent pneumococci had lain would itself neutralise the opsonin, the death of the guinea-pig being the result of the injection, along with the non-virulent cocci, of some of the virulent toxins in which they had been placed. He explicitly states that the washing of these cocci was "rapid." It does not seem to be necessary to postulate a specific "virulin" to explain the result.

The importance of this point has led us to undertake some experiments on the same lines with the meningococcus, since this organism resembles the pneumococcus, in that a presumably virulent strain is not susceptible of being taken up by the leucocytes.

We can confirm Rosenow's finding up to a certain point, that a non-virulent indigestible meningococcus strain, when grown in glucose-serum broth to which a certain quantity of a killed freshly isolated non-indigestible meningococcus culture extract had been added, is no longer taken up by the leucocytes. We prefer to ascribe our finding, however, to a neutralisation of the opsonic properties of the serum, by the fragments and debris of the killed extract. A certain amount of this debris had been taken up by the leucocytes. Moreover, if Rosenow's contention is correct, that virulence depends on a specific "virulin" and that this "virulin" is taken up by the non-virulent pneumococci, so that they are now transformed into proper strains, that are not taken up by the leucocytes; then if this quality is in any way similar to that found under natural conditions, it should be retained by these cocci on subculture. This, however, did not hold in the case of our meningococci. The ingestible condition was immediately lost on the first subculture. It is clear that the treatment they underwent did not in any way invest them with a virulence similar to that of the freshly isolated meningococcus, which invariably retains its ingestible condition through a number of subcultures.

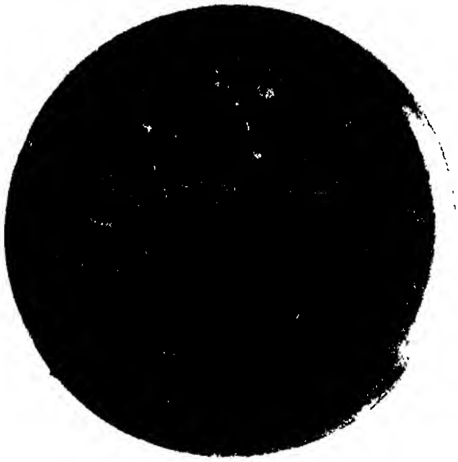

FIG. 1.

FIG. 2.

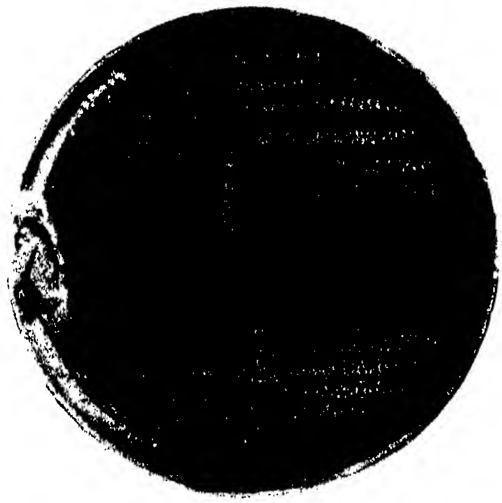

FIG. 3.

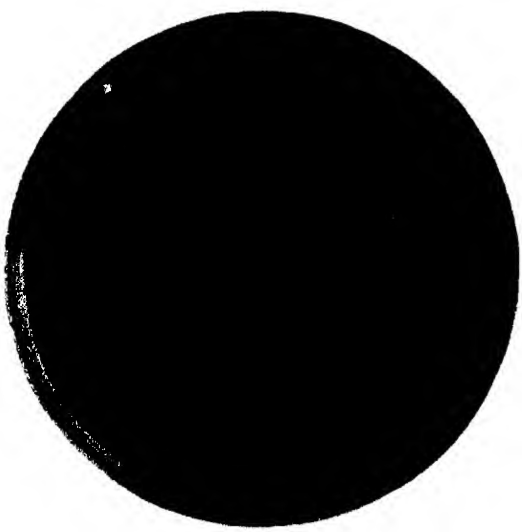

FIG. 4.

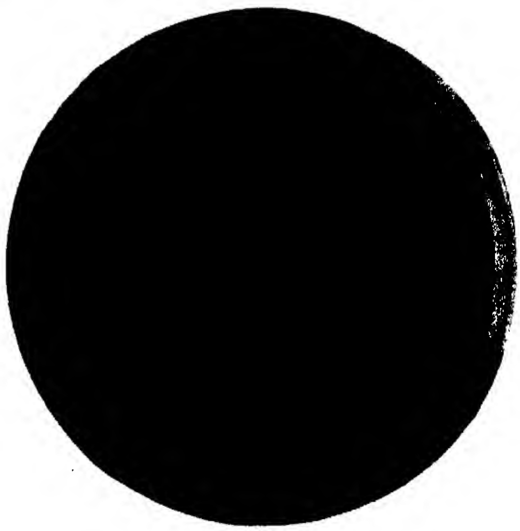

FIG. 5.

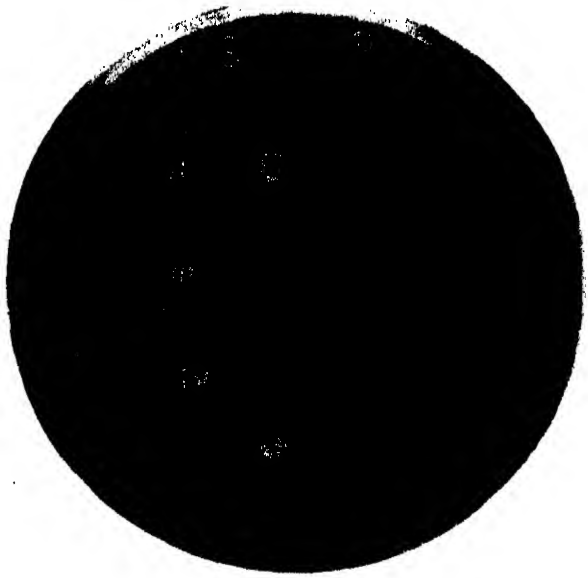

FIG. 6.

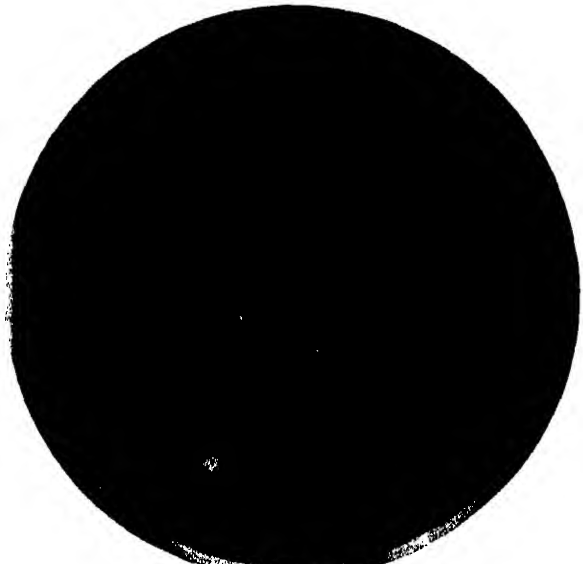

FIG. 7.

FIG. 8.

DESCRIPTION OF PLATES.

Experiment 3.

Fig. 1.—Photograph of planted out leucocytic deposit (1) of spinal fluid **washed four times in** 1 per cent. sterile glucose, after 24 hours' incubation at 37° C.

Fig. 2.—Photograph of planted out leucocytic deposit (2) of spinal fluid **washed 16 times in 0·85 per** cent. NaCl, after 24 hours' incubation, at 37° C.

Fig. 3.—Photograph of plate after 24 hours' incubation, to show the action of glucose on the growth of the meningococcus. Upper half of the plate shows eight dilutions of the germ in 1 per cent. glucose, while the lower half shows the same number of dilutions in distilled water.

The top and bottom line show the highest dilutions in each case.

Experiment 4.

Fig. 4.—Photograph of plate with leucocytic deposit of washed spinal fluid planted out immediately. In upper portion of plate the deposit has been planted out untouched. In the lower, the deposit has been crushed with sterile glass powder. Growth about the same on both portions of the plate, possibly slightly greater on the crushed area.

Fig. 5.—Photograph of leucocytic deposits similar to the last, but in this case they have been treated with 0·85 per cent. NaCl for three hours before being put on the plate. The upper portion of the plate represents the uncrushed while the lower shows the crushed deposit. Four or five colonies are showing after 24 hours' incubation in the upper half, while only one has developed in the lower half of the plate.

Experiment 5.

Fig. 6.—Photograph of plate used in Experiment 5, showing growth obtained at the end of 24 hours' incubation at 37° C.

Fig. 7.—Photograph of the same plate at 48 hours' incubation.

Fig. 8.—Microphotograph × 1000 showing in centre a large polymorph cell from freshly drawn spinal fluid being burst open by growing meningococci.

On the Toxic Action of Dilute Pure Sodium Chloride Solutions on the Meningococcus.

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(Received October 11, 1916.)

(Report to the Medical Research Committee.)

[Plate 15.]

In experimenting during the past season with a large number of freshly isolated strains of the meningococcus, it was noticed that almost all of these were killed, when placed for a short time in dilute pure sodium chloride solutions. This action of sodium chloride is most toxic to the meningococcus when the concentration of the salt is not below 0·85 per cent., and not much above 0·9 per cent. NaCl.

It was found that freshly isolated meningococci were more vulnerable to this action of NaCl, than old laboratory cultures. While old laboratory cultures could sometimes resist the action of a pure 0·85 per cent. NaCl for three or four hours, freshly isolated strains seldom resisted its action for more than 20 minutes.

It seemed remarkable that NaCl should be toxic for the meningococcus in just that concentration which it is accustomed to in the fluids of the body. It is strange that this germ should be so sensitive to the toxic action of sodium chloride, while at the same time it is able to resist for many hours the action of distilled water.

To demonstrate the toxic action of a physiological saline solution on the meningococcus, it is highly important to observe certain conditions in performing the experiment. It is essential, in the first place, that the NaCl used should be free from any impurity. In the second place, the meningococci must be added to such a quantity of the saline solution that any traces of salts brought over with them from the culture medium will have no appreciable effect in antagonising the action of the NaCl. Thirdly, it is very important that the saline is not unduly diluted below its proper toxic strength by the addition of too large a quantity of the emulsion containing the germs. In the fourth place, no agglutination of the germs into masses or clumps in the saline must take place. The saline is unable to act on the germs in the interior of these clumps, so that all are killed. Care must be taken to shake up the germs in the saline thoroughly, and avoid all clumping as much as possible.

I have found from a large number of experiments that one or two drops of an emulsion (of 5000 million meningococci to the cubic centimetre in

distilled water) is the proper quantity of emulsion to be added to 2 c.c. of 0·85 per cent. saline to effectively demonstrate the toxic action of pure NaCl on the meningococcus.

The toxic action of a 0·85 per cent. NaCl solution on the meningococcus can be readily antagonised and rendered harmless by the addition of a very small trace of some bivalent salt, such as $CaCl_2$, with or without the addition of a small quantity of KCl. This is clearly shown by the following experiment, which has been repeated many times.

A twenty-four-hour culture of a recently isolated strain of meningococcus "Lake" was emulsified. in distilled water; a fairly thick emulsion being prepared (about 5000 million cocci to the cubic centimetre). Into four sterile test-tubes, capable of being placed in a centrifuge, the following solutions, with 25 cu. mm. of the meningococcus emulsion, were placed as follows:—

1. 2 c.c. sterile 0·85 per cent. NaCl.
2. 2 c.c. " 0·85 " NaCl + 0·004 c.c. M/1 $CaCl_2$.
3. 2 c.c. " 0·85 " NaCl + 0·004 c.c. M/1 $CaCl_2$ + 0·01 c.c. M/1 KCl.
4. 2 c.c. " distilled water.

Each tube was then thoroughly shaken to ensure thorough mixing of the solutions and the emulsion of cocci. They were put in the incubator at 37° C. for an hour and a quarter. They were then taken out and centrifuged hard for 15 minutes, and the deposit in each tube planted out separately, in sterile fashion, on a chocolate plate.* Fig. 1 shows the growth obtained on this plate after incubation for 24 hours at 37° C.

An examination of fig. 1 shows that the meningococcus emulsion placed in the pure 0·85 per cent. NaCl, that is the deposit from the tube 1, has failed to grow, and that this quarter of the plate (marked N.S.), planted out with this deposit, is quite free of colonies. The cocci have been killed by the saline. In the opposite quadrant of the plate to this (marked II on the margin of the plate), which has received a similar quantity of emulsion in 0·85 per cent. NaCl, with the addition of a trace of $CaCl_2$, a thick heavy growth of the meningococcus has taken place, covering the entire surface of this quarter of the plate. The $CaCl_2$ here has completely antagonised the toxic action of the NaCl. In quadrant III, where the saline has received the same quantity of $CaCl_2$ as II, but also a little KCl, growth is still thicker (not very well shown in the photograph). In IV, where the germ was simply allowed to stand for an hour and a quarter in distilled water, growth is good, and the colonies cover closely the entire surface of this quarter of the plate.

* Crowe's "chocolate" or blood-trypagar-glucose medium. See 'Lancet,' November 21, 1915.

This experiment clearly demonstrates the four following points :—

1. The toxic action of a pure 0·85 per cent. NaCl solution on the meningococcus.
2. The antagonistic action of a trace of $CaCl_2$ solution over the toxic action of the NaCl.
3. The accelerating action of KCl, when added to $CaCl_2$, in antagonising the toxic action of NaCl.
4. The relatively harmless action of distilled water on the meningococcus.

So definite is this toxic action of 0·85 per cent. NaCl solution on the meningococcus, that it was found possible to make use of it, very successfully, to destroy all meningococci outside or attached to the surface of leucocytes, by simply washing these several times and allowing them to stand for a few hours in a small bulk of pure saline.*

There is no doubt that in this toxic action of dilute NaCl solution on the meningococcus we are dealing with the poisonous action of the Na cation, so extensively investigated by Loeb,† Wasteneys,‡ Osterhout,§ and others.

It is interesting to find that in the case of the meningococcus, as these investigators have found for other forms of life, this toxic action of NaCl is confined to relatively dilute solutions. In the case of the meningococcus, it is essential that the concentration of the NaCl should not be increased much beyond 0·9 per cent., as after this point its toxic action rapidly decreases. The use of a 1·5 per cent. NaCl solution (one of the standard strengths of this salt employed in opsonic work) is without almost any toxic action on the meningococcus, as shown by the following experiment:—

A fairly thick emulsion of a 24 hours' culture of meningococcus "Pryor" was made (about 5000 million cocci to the cubic centimetre). To 4 c.c. of a pure 1·5 per cent. NaCl solution, 25 cu. mm. of this culture, in distilled water, was added, and thoroughly mixed. To 4 c.c. of 0·85 per cent. NaCl solution a similar quantity of the same emulsion was added and mixed. The two solutions were placed in the incubator at 37° C. for an hour. They were then taken out and centrifuged, and the deposit planted out separately on the surface of a chocolate plate, as shown in fig. 2. This figure shows the resulting growth obtained on this plate after 24 hours' incubation at 37° C.

The 0·85 per cent. NaCl solution (marked N in the plate) has killed the meningococcus, while a good growth has been obtained on that half of

* See paper by Shearer and Crowe, "The *Rôle* of the Phagocyte in Cerebro-spinal Meningitis," 'Roy. Soc. Proc.,' B, vol. 89, p. 422 (1916).

† Loeb, 'Collected Papers,' Part II, University of Chicago, 1906.

‡ Loeb and Wasteneys, 'Journ. Bio. Chem.,' vol. 21 (1915).

§ Osterhout, 'Zeit. f. Physkl. Chem.,' vol. 70 (1910).

FIG 1.

1.5% NaCl Sol.

0.85% NaCl Sol.

FIG. 2

FIG. 3.

the plate inoculated with a similar quantity of the same emulsion of the meningococcus, which had been exposed to the action of a 1·5 per cent. NaCl solution. Thus, as Loeb has found, when the concentration of the NaCl solution increases the toxic action diminishes.

I should like to draw attention, finally, to an experiment made to determine the length of time the meningococcus may remain alive in pure distilled water, as compared with the time it can remain alive in a 0·85 per cent. NaCl solution.

Into 4 c.c. of distilled water and 4 c.c. of 0·85 per cent. NaCl solution respectively, 25 cu. mm. of a meningococcus emulsion was placed. The two solutions were incubated for 24 hours at 37° C., centrifuged down for an hour, and planted out separately on a chocolate plate. In fig. 3 is shown the resulting growth on this plate after 24 hours' incubation at 37° C. The saline, as usual, has killed the germs, while a considerable number of those that have been exposed to the action of the distilled water for 24 hours have survived, and have given rise to an extensive growth. This experiment clearly demonstrates the power of the meningococcus to resist the hypotonic action of distilled water for many hours.

This experiment has been repeated with a large number of different strains of the meningococcus, and it was found that considerable difference in this power of withstanding the action of distilled water was possessed by each strain. It was the exception, however, to find a strain which did not survive the exposure to the action of distilled water at 37° C. for three hours.

DESCRIPTION OF PLATE 15.

FIG. 1.—Photograph of the growth obtained on a plate of Crowe's chocolate medium after 24 hours' incubation at 37° C., showing the toxic action of a pure 0·85 per cent. NaCl solution on the meningococcus and the antagonistic action of a trace of $CaCl_2$. I. Portion of plate planted out with emulsion of the meningococcus in 0·85 per cent. NaCl solution. No growth whatever has taken place, all organisms being killed. II. Portion of plate planted out with a similar quantity of emulsion in 0·85 per cent. NaCl + a trace of $CaCl_2$; here the $CaCl_2$ has antagonised the toxic action of the NaCl, with a resulting thick growth of the meningococcus. III. Portion of plate planted out with a similar quantity of emulsion in 0·85 per cent. NaCl + $CaCl_2$ + KCl, with a resulting thick heavy growth, more than in II. IV. Portion of plate planted out with a similar quantity of meningococcus emulsion which had been allowed to stand for 1½ hours in distilled water; good growth.

FIG. 2.—Showing the toxic action of 0·85 NaCl solution on the meningococcus, in distinction to the relatively harmless action of a 1·5 per cent. solution of the same salt. N. Normal saline solution.

FIG. 3.—Showing the action of distilled water in failing to kill the meningococcus after 24 hours. D. Distilled water portion. N.S. Saline portion of the plate; all germs killed.

Croonian Lecture: *The Respiratory Process in Muscle and the Nature of Muscular Motion.*

By Dr. W. M. Fletcher, F.R.S., and Prof. F. G. Hopkins, F.R.S.

(Lecture delivered December 9, 1915.—MS. received November 22, 1916.)

Mr. President and Fellows:—We are keenly sensible of the honour done to us in our being called to lecture on this occasion, and in making this acknowledgment we would express our special gratification in being so enabled to pay this act of piety to the memory of William Croone, whom we commemorate to-day. The Croonian Lecture was founded through his generosity in order to encourage the study of muscular motion, but some sixteen years have now passed since that subject was last treated by the Lecturer. During those years many additions have been made to our knowledge of the subject, and great changes have resulted in our views of it. It is a pleasure to us that we have now the opportunity of taking up again the broken thread of the series, and of turning to-day to the chosen subject of Croone's own enquiries and chief interest. We could wish that a time more free from other occupations and anxieties than the present had allowed us to do this less unworthily.

Croone found in muscle the chief immediate hope of studying the energy discharges of living elements, and it was surely an enlightened instinct which led him to foresee, however dimly then, what we must recognise as still true after this lapse of two and a half centuries. We still must look to the study of muscular motion as the most fruitful, and perhaps for some time to come the only, avenue to intimate knowledge of the modes of energy discharge by the living cell, and of their relation to the specific chemical processes of life. More than this, it is the study of muscle activity which has so far given us all we know of the meaning of respiration as the accompaniment of life. The study of respiratory exchanges in the lungs and in the blood of mammals has given us valuable lessons, and has unfolded attractive stories of animal adaptation to environment. That study takes its place in the natural history of the Vertebrates, and has a living value for the purposes of human medicine. It is describing to us the modes in which oxygen reaches and carbon dioxide leaves the cell under the anatomical conditions of the vertebrate animal, but it does not attack the intimate problems of respiration as a process of animal cell life in general. Croone, of Cambridge, was too close in time and sympathy to the genius of Mayow, and to the work of his other contemporaries at

Oxford, not to realise that in the study of muscle lay probably the first path to knowledge of the inner processes of life within the living substance itself.

Intramolecular Oxygen and the Theory of "Inogen."

The closing years of the nineteenth century, and with them the last occasion on which the Croonian Lecturer dealt with the subject prescribed by the endowment, marked what seemed the final establishment of a particular conception of respiration in muscle. It was almost universally held that muscular energy and, by inference, the energy liberated in any cell upon activity, whether as mechanical energy or heat, sprang from a more or less explosive splitting of a molecular complex which had been made highly unstable, that is "irritable," by the previous inclusion within it of oxygen taken in by the cell during rest. The breakdown of this hypothetical molecule was supposed to yield both lactic acid and carbon dioxide, these being the two obvious and recognisable products of activity.

The earliest phases of this conception, through all the stages of the long-delayed discovery of oxygen, connected the idea of "irritability" directly with that of combustibility. This notion, however, was negatived by the discovery of Spallanzani that living tissues could long survive and continue to yield carbon dioxide without any supply of oxygen except such as had previously been available. This yield of carbon dioxide in the contemporary absence of oxygen was shown to be true for the case of isolated muscle by Müller, Liebig, Matteuci, and others, and it was upon this observation that Hermann chiefly based his theory of inogen. He showed in 1867 that free oxygen was not present in the air pumped from isolated frog's muscle, and yet he found that without any fresh oxygen supply from outside, carbon dioxide was yielded by the muscle when it contracted or when it stiffened after death. At the same time, lactic acid was produced, as had previously been shown, while no nitrogen bodies could be recognised as appearing.

Hermann's hypothetical "inogen" accordingly was the unstable precursor of both lactic acid and carbon dioxide, a precursor in which oxygen was already combined, or placed in a position to combine, with carbon and hydrogen in the combustion which was to yield the energy of contraction. After the explosive breakdown of this precursor, it was supposed that fresh carbon bodies, and perhaps also the lactic acid, were combined again in a newly oxygenated unstable molecule of inogen.

This inogen hypothesis of Hermann was taken up again ten years later by Pflüger in his well known studies of "physiological combustion." Here they were amplified and illustrated with great wealth of rhetoric, but without significant change and without fresh experimental support. Pflüger's

"giant" molecule, as he described it, made unstable by the inclusion within it of what he called "intramolecular oxygen," was the same in all essentials as the inogen molecule of Hermann.

These conceptions of Hermann and Pflüger have had an historical importance reaching far beyond the particular enquiry into muscular energy. They summarised the only aspects of cell metabolism which had received any experimental analysis at all, and up to the end of the nineteenth century they not only represented all that was known of cell respiration and of its relations to cell energy, but they dominated also all our ideas of cell metabolism in general. It was conceived that the chemical processes of life in all cells consisted essentially in the building up of elaborate, unstable, and oxygen-charged molecules, by the processes of so-called "anabolism," into the mystical complexes of irritable protoplasm. From protoplasm, as seen in chemical imagination, a descent by the stages of so-called "catabolism" was conceived to follow, by which through successive splitting processes energy was discharged, and certain recognisable end-products were displayed.

Michael Foster, a name familiar and loved in this place no less than in Cambridge, wrote in 1895 as follows:—

"The oxygen taken in by the muscle, whatever be its exact condition immediately upon its entrance to the muscular substance, in the phase which has been called 'intramolecular,' sooner or later enters into a combination, or, perhaps we should rather say, enters into a series of combinations. We have previously urged that all living substance may be regarded as incessantly undergoing changes of a double kind, changes of building up, and changes of breaking down. . . . We cannot as yet trace out the steps taken by the oxygen from the moment it slips from the blood into the muscular substance to the moment when it issues united with carbon as carbonic acid. The whole mystery of life lies hidden in the story of that progress, and for the present we must be content with simply knowing the beginning and the end."*

The story of that progress is part of the story we have to tell to-day, and these words of Foster may be taken as the summary of what was the current physiological opinion some eighteen years ago, when the work now to be discussed began at Cambridge.

We must note first that the inogen theory had two main bases of experimental support. These were:—

(1) The contraction of muscle and the death of muscle alike were believed

* 'Text Book of Physiology' (Sixth Edition), Book II, p. 610.

to give a simultaneous fresh production of lactic acid and of carbon dioxide in the absence of an immediate oxygen supply. The introduction of oxygen and the preparation for combustion had taken place beforehand.

(2) The instability of the inogen molecule increased with rise of temperature, and so also the rate of production of lactic acid and carbon dioxide, but it was believed that, if the muscle were scalded suddenly with boiling water, the molecule could be "fixed" without a yield either of lactic acid or of carbon dioxide.

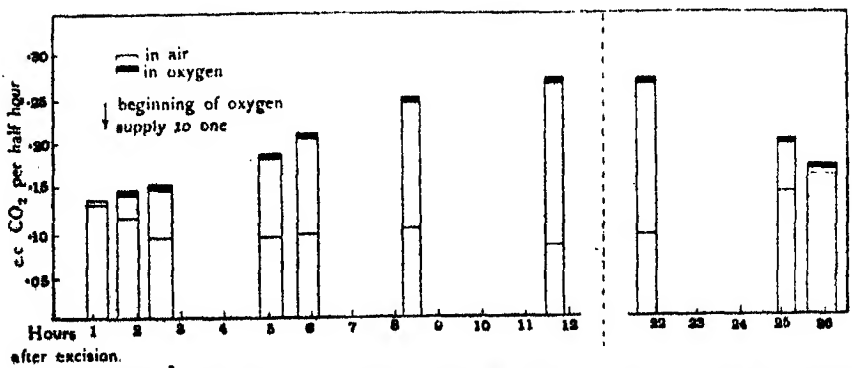
The evidence under both these heads was first examined by one of us so far as the carbon dioxide production was concerned (1).

It will be enough now to recall that in this work improved titration methods were used for the estimation of carbon dioxide, in place of Hermann's eudiometric method, and that the new knowledge of bacterial action allowed the results of early putrefaction to be recognised and left out of account.

By successive estimations, the course of carbon dioxide discharge from isolated frog's muscle was followed. At rest the muscle gave a high initial rate of discharge, which soon descended to a lower rate, maintained at steady level for many hours.

When the muscle was stimulated to contract, an outburst of carbon dioxide such as Hermann had found, and as all the text-books of the day described, was expected and looked for, but none was found; no increase of carbon dioxide accompanied contraction unless the contraction was forced by repeated strong stimulations to give marked fatigue.

At rest again, the muscle in nitrogen gave a lower steady output of carbon dioxide than in air; in oxygen it yielded carbon dioxide two or three times as fast (fig. 1).



1.—Survival discharge from "crossed" pairs of legs, one in air, the other in oxygen. From the 'Journal of Physiology,' vol. 28, p. 354 (1902).

On contraction in oxygen, and now even on slight contraction, it yielded the increase of carbon dioxide expected by the text-books to be shown in air [(3) and fig. 2.]

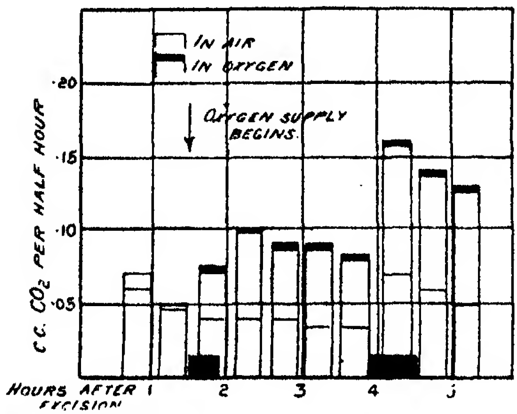


FIG. 2.—Course of survival discharge of carbon dioxide from two "crossed" pairs of gastrocnemii. Contraction periods are shaded. Temp. 17° C. (In the first contraction period slow rhythmic stimuli were given and fatigue was not shown; in the second period, rapid stimuli were given and the muscles were fatigued to a standstill.) For details see the 'Journal of Physiology,' vol. 28, p. 474 (1902).

It may be said in passing that these results accorded with many observations made previously upon the whole animal in which conditions of imperfect oxygen supply had given apparent incompletion of oxidative processes, and explained many earlier discordant observations made when blood was circulated through muscle.

It became clear then that the contemporary and immediate supply of oxygen did affect the products due to contraction, and the inogen theory, postulating a previous inclusion of oxygen within the muscle elements, was evidently inadequate.

We now come to the second of the two pillars of the inogen hypothesis—the effects of heat upon muscle and the supposed "fixation" of inogen by rapid scalding. The close historical dependence of the hypothesis upon the experimental results of heating has not, we think, been sufficiently recognised.

Du Bois Reymond had made the observation that a muscle if slowly killed by heat became markedly acid, but not if it was rapidly killed by scalding. Hermann, in his view of inogen, assumed that lactic acid and carbon dioxide found in it their common and simultaneous source of origin, and, probably biassed by this, he claimed to show experimentally that scalded muscle yielded not only no lactic acid, but also no carbon dioxide; but in fact, though this simply tested phenomenon became the commonplace of the text-

book and has so remained almost to this day, the evidence given by Hermann's eudiometric experiments cannot be accepted on examination.

It was long ago shown at Cambridge (1) that a large volume of carbon dioxide is expelled from the muscle if rapidly scalded, though it was shown also later (5) that, as du Bois Reymond had found, practically no lactic acid is produced.

More recently it has been shown (17) that there are two sources of the carbon dioxide expelled on heating; one is the preformed carbon dioxide held probably in union as carbonate, which is displaceable by acid but not by heat, while the other is that held in firmer chemical union with protein groups, not displaceable by acid yet dissociable near the boiling temperature. On slow heating, with consequent acid formation, only the former source yields carbon dioxide; on rapid heating, without acid formation, and at the high temperatures (80—100° C.) necessary if heating is to be rapid enough, the firmly held carbon dioxide, and that only, is dissipated. Heating, as such, though it may produce a maximum yield of lactic acid, is not accompanied by any fresh production of carbon dioxide.

Pflüger (with Stintzing) also supported the origin of carbon dioxide from a previously oxygenated "giant" molecule by finding that after washing with acid to expel any previously formed carbon dioxide, the giant or inogen molecule broke down on heating to give what they believed to be, and called, "newly engendered carbonic acid." There were grave fallacies however in their technique, and, putting the matter as shortly as possible, when the trial was repeated at Cambridge by better methods it was found that acid applied to muscle at 0° C. expels the preformed carbon dioxide, that the muscle thereafter raised to 40° C. (when the maximal lactic acid yield is given) gives now no carbon dioxide, while heating further to 100° C. gives the normal amount for that temperature as from an untreated muscle (17). Pflüger had in fact been misled by his failure to recognise the double mode of storage of carbon dioxide in muscle. His "newly engendered" carbon dioxide was the carbon dioxide dissociable from muscle proteins on their coagulation at high temperatures, and it has no relation to the energy store of muscle.

The last historical support of the inogen theory then, that of the results of heating, breaks down. So far from lactic acid and carbon dioxide arising together from a common precursor, as Hermann and Pflüger taught, we see that only the particular conditions of experiment determine whether a carbon dioxide discharge appears to accompany lactic acid formation in the muscle or not.

In a muscle at rest in air, and more rapidly in a muscle in nitrogen, lactic

acid is continuously produced (5) and proportionate volumes of preformed carbon dioxide are expelled; as we saw earlier, the slow yield of carbon dioxide is steadily maintained for many hours from an isolated muscle (1). On contraction, and especially in nitrogen, acid production is faster, and the carbon dioxide is expelled faster (3). So we return to the ancient observation of Spallanzani that carbon dioxide is yielded (as he thought, freshly produced) without the immediate presence of oxygen, and, as we have seen, it was largely upon this supposed evidence of a previously oxygenated precursor of carbon dioxide in the muscle that the inogen theory was later to be erected.

The Effects of Oxygen upon Muscle.

According to the ancient view that the irritability and activity of a muscle depended upon its combustibility, oxygen would be expected to hasten the energy discharge by muscle, and so to act as a stimulant or irritant, very much as oxygen kindles glowing tinder to a flame. But on the inogen hypothesis as developed by Hermann, while oxygen should restore and maintain the capacity for energy discharge by completion of the oxygenation of the inogen molecule, it would not be expected to cause or to favour the explosive splitting of the molecule.

Humboldt in 1795, and many others after, had shown that isolated muscle maintained its irritability longer with an abundant oxygen supply than without, and was longer preserved from fatigue after stimulation. Hermann spoke with uncertain voice on this fundamental point. He claimed that oxygen was irritant and destructive at the surface of a muscle, hastening death, but that in bulky muscles exposed to it, it had a preservative action, maintaining irritability below the surface layers. But he used faulty methods, gave few actual data, and obtained obscure results.

With more appropriate methods it was found at Cambridge (2), as we have seen already, that in oxygen the carbon dioxide yield of the muscle was increased threefold or more (see fig. 1) and that, nevertheless, in spite of this increased combustion the irritability, as many from Humboldt to Joteyko had previously found, was not more quickly exhausted but longer maintained. All irritant gases increase the yield of carbon dioxide by quickening the production of the lactic acid, which expels preformed carbon dioxide held in the muscle. But oxygen, while it would set a combustion flaring, not only delays the stiffening of the muscle, but may altogether inhibit its onset. A muscle forced by stimulation to stiffening may be recalled again by oxygen to its previous flaccidity (3).

This seems to us to be a crucial experiment manifesting an immediate

oxidative removal of some product of activity which is a basis of fatigue and of stiffening, giving at the same time a yield of carbon dioxide as the obvious sign of a completed combustion.

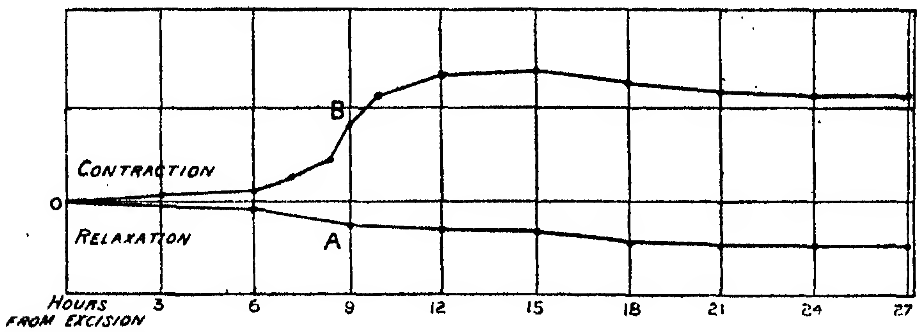


FIG. 3.—Changes in length of a pair of excised gastrocnemii, after fatigue. The ordinates are measured directly from the record upon the drum. The levers magnified 6½ times. Load 3 grm. Temp. 23° C. A. Exposed to oxygen. B. Exposed to air.

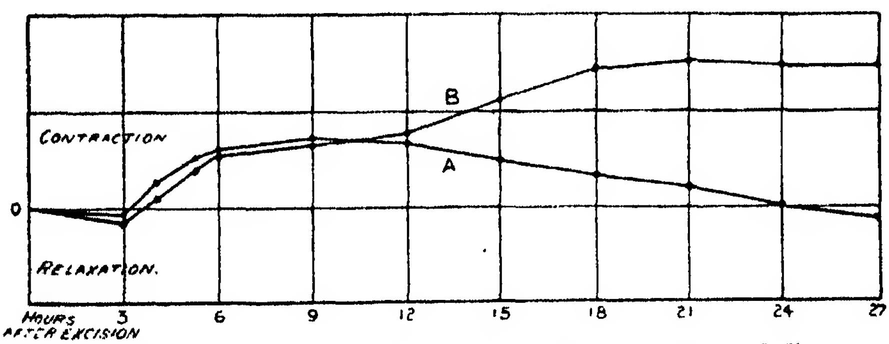


FIG. 4.—Changes in length of a pair of excised gastrocnemii, after fatigue. Ordinates and magnification as in fig. 3. Load, 3 grm. Temp. 16° C. A. Exposed to oxygen. B. Exposed to air. From the 'Journal of Physiology,' vol. 28, p. 479 (1902).

Now lactic acid itself is the most obvious determining cause of the signs both of fatigue and of the stiffening of rigor mortis. Its artificial application to muscle can mimic the signs of both (1). And, indeed, it had often been suggested from observations in the whole animal that lactic acid was a product of activity whose expulsion was effected by burning to carbon dioxide and water. One sign of the presence of lactic acid in fatigued muscle is to be found in a characteristic change of the osmotic properties of the muscle, and it was further shown at Cambridge that immersion of a fatigued muscle in oxygen restored the osmotic properties to those of resting muscle (4).

All these results pointed irresistibly to the conclusion that lactic acid

produced by muscle contraction or upon dying is oxidisable, or in some way removable by oxygen, with an accompanying production of carbon dioxide. The next step was to obtain direct evidence of the changes undergone by lactic acid in muscle. Lactic acid outside muscle, in the circulation for instance, is not directly oxidisable at physiological temperatures.

Lactic Acid in Muscle.

It is a remarkable fact that up to less than ten years ago we had little or no knowledge of the most elementary relations of this acid to the physiology of muscle. Production of free acid appears to be an almost universal sign of the activity of any living cell, and a sign also of the processes leading to death; but even in the conspicuous case of muscle nothing was known certainly with regard to the conditions of lactic acid production, save the fact of its happening. This is another striking instance of the slenderness of the foundation upon which the inogen hypothesis had been erected, and with it, as we have seen, almost the whole structure of prevalent ideas with regard to the general nature of the processes of metabolism.

The inherent difficulty besetting the chemical examination of muscle lies, of course, in the fact that the necessary processes for extraction of the constituents cause in the moment of their application profound chemical change. It will not be appropriate here to explain in detail the chief fallacies underlying the methods which had formerly been used. It may be said, however, that up to a few years ago there was hardly any single statement made with regard to the conditions of lactic acid appearance in muscle which was not both supported and contradicted by rival sets of observers respectively.

In our own work (5), of which we propose to give very shortly the chief results, we found that the disturbing influences introduced by the mechanical and chemical operations necessary to the process of investigation, could be reduced to a minimum if throughout the whole of their performance the muscle was maintained at a temperature close to the freezing point. Completely resting muscle examined in this way in the cold, when the cold is maintained until the extracting processes are complete and the muscle killed, gives only the smallest traces of lactic acid, and these traces must be attributed to the unavoidable minimum of manipulation before the low temperature is reached. Resting muscle, that is to say, may be regarded as muscle containing at most only traces of free lactic acid.

In order to determine the lactic acid production associated with any particular muscular condition, whether of fatigue or of spontaneous resting change, the processes of examination were carried out again only when the

temperature had been brought to the freezing point, by which the *status in quo* could be maintained.

The first qualitative estimations that were undertaken showed at the first attempt that fatigued muscle contained more lactic acid than resting muscle, and that fatigued muscle after resting in an oxygen atmosphere subsequently contained less lactic acid—a result which was confidently expected in view of the experiments which have been described already.

Attempts were then made to improve the technical methods for the accurate quantitative estimation of small quantities of lactic acid under the required conditions. In the end, and after trial of alternatives, resort was had to the old method of estimation by weight of the zinc salt obtained from the dextro-rotatory acid which muscle yields, and in the details of this method certain improvements were effected.

The chief facts relating to the production of lactic acid in the muscle substance, as these have been determined by our estimations, may be shortly stated.

Mechanical injury, like that of chopping up the muscle, produces a rapid increase of lactic acid. This rate of production is accelerated by rise of temperature, and is brought to a standstill at the freezing point.

Isolated undamaged muscle left at rest in air at ordinary temperatures continues to yield lactic acid, so that the total acidity progressively and steadily increases for many hours. Outward signs of this acid production are found in visible physical changes of the muscle, as shortening, stiffening, and loss of translucency, and it is accompanied by a corresponding yield of carbon dioxide previously held in the muscle, but now expelled by the increasing acid.

As the temperature increases, this spontaneous yield of lactic acid is accelerated. Between 35° C. and 40° C. it is very rapid and reaches a maximum almost instantaneously. This is the "acid maximum" formerly described by Ranke. Nevertheless, if the muscle be rapidly scalded, the source of lactic acid is "fixed," and little or no acid production takes place, as du Bois Reymond formerly showed.

If the muscle be left at room temperatures in nitrogen or other anaërobic atmospheres, it yields lactic acid at a uniform rate determined by the temperature, and so approaches and finally reaches the acid maximum. It reaches it, however, faster than it does in air at the same temperature. In oxygen, on the other hand, it is found to accumulate no lactic acid at all during many hours or indeed during days at room temperatures.

Upon stimulation an increase of lactic acid is found. This had been known of course from du Bois Reymond's time, but the fact had been repeatedly

denied, and the actual quantitative evidence supporting it had been wholly unsatisfactory.

If a muscle so fatigued, and containing lactic acid, be now left at rest in an

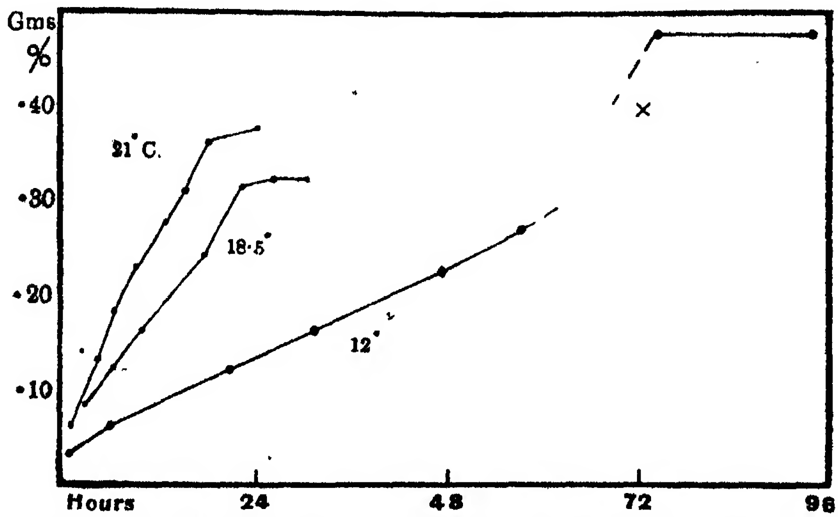


Fig. 5.—The course of lactic acid production in an atmosphere of hydrogen at 12° C. (Figs. 5, 6, and 7 from the 'Journal of Physiology,' vol 35, p. 273 (1907).)

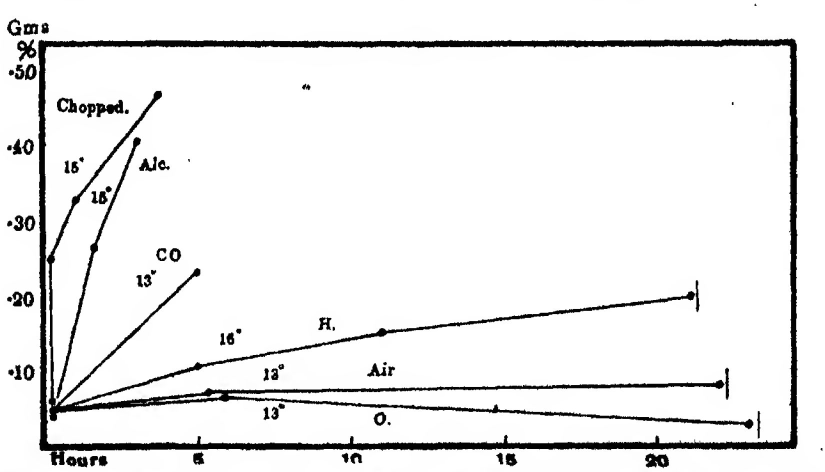


Fig. 6.—The course of lactic acid production occurring during survival periods in oxygen, air, hydrogen, and coal gas (CO) respectively. The two uppermost curves are introduced for comparison of those for chopped muscle and for alcohol immersion.

oxygen atmosphere, a notable decrease of lactic acid occurs. The acid is diminished quickly at first and later more slowly. It disappears, just as we saw earlier that fatigue or the stiffening of early rigor mortis disappears, when

the fatigued or dying muscle is placed in oxygen. With a rise of temperature above 30°, however, the accelerated spontaneous production of lactic acid overcomes this oxidative removal; the muscle enters into heat rigor and develops the acid maximum in spite of the presence of oxygen (fig. 7).

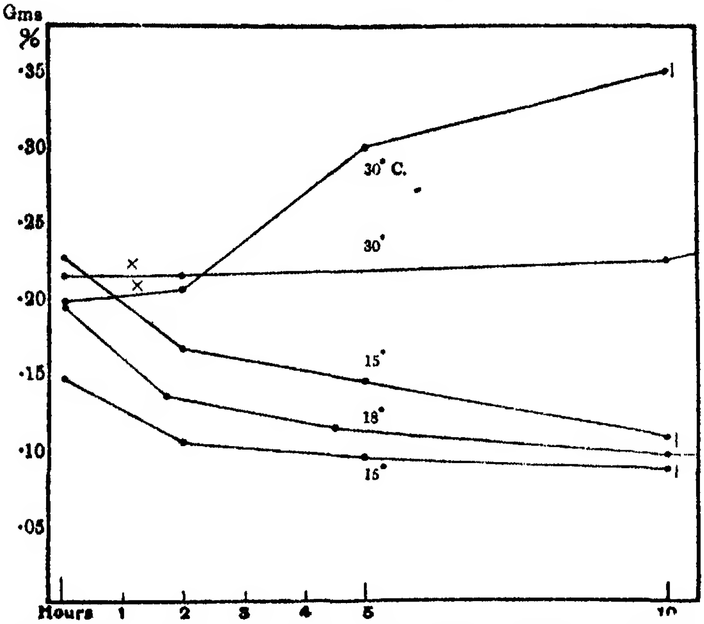


FIG. 7.—Lactic acid production and loss, in atmosphere of oxygen at different temperatures. Fatigued muscles were used for all. At 30° C. gain in lactic acid is shown: at 15–18° C. the course of loss is followed. × loss of excitability.

Severe mechanical injury, moreover, produces an acid yield which is unbalanced by oxidative removal, and muscle chopped in pieces or ground up with sand in the presence of oxygen very rapidly reaches the acid maximum, and reaches it apparently as rapidly as if oxygen were absent. It seems that the normal architecture of the muscle is a necessary condition for the oxidative process of removal.

These results as here stated very shortly, when set side by side with the facts of the carbon dioxide output already given, show clearly again that oxygen does in fact enter the living substance of muscle for the purposes of an immediate oxidation, and not as a preparer or builder up of material ready for explosion. We are now therefore in a position to distinguish more clearly those chemical events in muscle which are anaërobic and independent of oxygen, from those, on the other hand, in which oxygen plays a part.

Plainly the act of contraction and the process of rigor, each with its accompaniment of lactic acid formation, are anaërobic functions. Neither of them, it

is to be emphasised again, is associated with any fresh yield of carbon dioxide, except such as is directly due to expulsion, by the lactic acid, of previously formed carbon dioxide loosely combined in the muscle.

In the oxygen atmosphere, however, we have a removal of acid, a simultaneous yield of newly-formed carbon dioxide, with a restoration of the *status in quo ante* and of the previous "potential" of the muscle. Carbon dioxide production in an atmosphere of oxygen is a sign and a measure of an immediate contemporary combustion.

The Heat-Production of Muscle.

These results, it must be pointed out, are the results of the chemical study of an integrated series of contractions in a muscle. The present limitations of chemical method do not allow us to measure and follow the time relations of the relatively minute changes which accompany and succeed each single act of contraction.

During recent years, however, Mr. A. V. Hill has conducted, at Cambridge, a long series of investigations into the heat production of muscle by means of the most refined thermo-electric methods. Of this work we must not pretend here to give any adequate account, but we must notice in general that, following up the results of our own experimental and chemical work, and using the same general experimental methods of analysis of the conditions of fatigue, of rigor (whether inflicted by heat or by chloroform), of recovery in oxygen, and so on, Mr. Hill has obtained a valuable series of parallel observations of heat production which have fundamental importance for the theory of muscular metabolism. By ingenious modifications of the thermopile and with a highly sensitive galvanometer Mr. Hill has been able to record the temperature changes associated with a single act of contraction, and so by exposing the muscle either to nitrogen or to oxygen the anaërobic heat production can be distinguished from the aërobic.

He finds that if the muscle contracts after being an hour or more in nitrogen, the heat production observed as the accompaniment of contraction does not continue beyond it (12). In oxygen, however, the heat production of contraction is continued for long periods after the mechanical event is over. The amount of heat liberated during the recovery process in oxygen he found to be at least as great as that due to the anaërobic act of contraction itself.

We must not now stay to speak of the many other general respects in which Mr. Hill's work has confirmed and further illuminated our own observations made on the chemical side.

Before passing, however, to some general considerations, we would draw attention to the fact that for important reasons of technique (which we must

not now discuss) all these experimental results at Cambridge have been gained by the use of muscle isolated from a cold-blooded animal. We have already, however, sufficient assurance that in all essentials the results can be taken to apply equally, *mutatis mutandis*, to the case of warm-blooded mammalian muscle (16). Verzar, at Cambridge (9), Winterstein and others abroad, have also confirmed our results with amphibian muscle by showing them again in the case of mammalian muscle.

The considerations which have so far been brought forward seem to lead to a conclusion from which there is no escape. The special processes which, when they occur within a muscle fibre, culminate in a contraction, make no call upon an oxygen supply; they proceed anaërobically. The oxidations which are always associated with muscular activity are separated in time from that moment in which mechanical energy is liberated. They occur immediately afterwards, and are concerned not with the induction of the mechanical act, but with a restoration to the *status quo ante*. They are concerned not with stimulation but with recuperation.

Our problem, then, is to find a full description for each of these two phases of change within the muscle, the anaërobic leading to contraction, the oxidative resulting in recovery.

We are concerned in this Lecture in the main with the respiratory or oxidative phenomena, but in dealing with these we must, of course, have regard to the muscular act as a whole. Here we would beg in advance your indulgent consideration; the main problems before us, as we have urged already, lie at the centre of what knowledge we have of the processes of cell life. They lie close at every point along their borders to other great fields of physiological enquiry. Their full, or, indeed, their adequate, discussion should involve reference to manifold considerations in regard to the facts of general metabolism, to questions of chemical energy and its transformations, to the phenomena of electrophysiology, and to the intricate problems of colloidal molecular physics. In the brief scope of one lecture, we can only attempt to point to the considerations which arise more immediately from the experimental results we have given.

In the first place, for the sake of greater clearness, it will be well to point at once to the provisional conception we adopt of the part played in muscular motion by lactic acid itself. So far from this being regarded as a toxic product to be eliminated as rapidly as possible, there is abundant reason supplied by many lines of converging evidence for seeing in lactic acid an essential agent in the machinery of contraction itself. The development of acid, with free H-ions, in the neighbourhood of colloidal fibrils gives the condition for contraction, whether by increasing the

molecular tension along longitudinal surfaces, or whether by the process of imbibition, causing a resultant increase of tension in the fibre. A catgut fibre in water will contract if its temperature be raised, as Engelmann showed here in his Croonian Lecture of 1895, and it will contract if acid be brought to it, relaxing again on its removal. We know that artificial application of lactic acid to muscle causes contraction, reversible by removal, as one of us showed long ago (1), and we have noticed to-day the shortening produced in muscle as lactic acid accumulates after fatigue and the lengthening which follows its removal by oxygen (Fig. 4). Engelmann thought of muscle as a heat engine, but we know now that, apart from other theoretical objections to that view, the heat production of contraction may take place after the mechanical contraction is over, and, in physiological conditions with oxygen present, the greatest heat production is always subsequent in time to the contraction. But, in spite of this dislocation in time, there is, as Hill has shown (7), a constant ratio between the new tension set up in the contractile elements and the heat of the contraction. The heat of the contraction, however, will be proportionate to the chemical reaction yielding it, so that we may say that there is a constant ratio between the increased fibrillar tension and the new chemical condition causing it, and regard the muscle as a chemical instead of as a heat engine; this, indeed, very many other considerations, into which time now forbids us to enter, force us to believe. Such a chemical event, proportionate to heat production, and also to the new tensile stress, would be the appearance, close to the muscle-fibrils, of the H-ions of lactic acid as this arises from some forerunner. The new condition of elastic state of the fibrils will give contraction if the mechanical conditions allow it, and work will be done according to the opposition given during the contraction. The work actually done, however, will bear variable and quite accidental relations to the heat production, as we have long known that it does, and the mechanical efficiency of the machine will vary accordingly with the conditions. The removal of lactic acid under the influence of oxygen will give relaxation, as the original state of tension in the fibril is restored.

To complete the image before us, we have still to consider the nature and the results of this oxidative removal of the lactic acid, hoping to reconcile in hypothesis the ascertained facts with regard to energy exchanges and chemical events.

On the simplest view, we might picture the muscle fibre as endowed initially with a supply of a substance (probably derived from carbohydrate) capable of yielding lactic acid by a non-oxidative molecular rupture. This rupture is exothermic, and heat is yielded proportionately to the acid

formed, and to the new surface tension or elastic tension imposed on the fibril by the acid ions. If the lactic acid be allowed by repetitions of the process to accumulate, fatigue phenomena are produced, and on this view fatigue is the expression, not of an exhaustion of energy supply, but only of a clogging of the machine. With a normal oxygen supply, however, the lactic acid is promptly removed after each contraction, and each successive stimulus, with its associated breakdown, is followed by a normal contraction. The removal of the lactic acid might be thought of as a direct oxidation—in the presence of oxidases—and it might be supposed to be burnt, so to speak, to waste, when the energy liberated by its combustion would supply nothing to the mechanical energy of contraction.

But this simplest view we are driven at once to forgo, and perhaps not unwillingly, since it would be unwelcome to believe that a body of such high energy value as lactic acid can be only a waste product yielding nothing in its discharge except the indirect benefits of heat production unconnected with the muscle machinery. Direct observation assures us, however, that of the energy of combustion of the lactic acid part at least remains in the muscle, for the work at Cambridge, both of Hill and more lately of Parnas (20), though they differ in other vital respects, concurs in this, that the energy leaving the muscle as heat in the oxidative removal of lactic acid is less than that calculated for the combustion in the oxygen used up. Some of the energy of that combustion is restored in some shape and to some degree at least to the muscle system.

On this account the general trend of opinion based upon the recent work we have described has returned to an old conception tentatively offered by Hermann, who suggested that in the building up of the inogen molecule there might enter again, with oxygen, part of the lactic acid from the previous contraction, there to be arranged in the explosive complex from which at the next contraction lactic acid and carbon dioxide should emerge.

There are grave theoretical difficulties associated with the conception of an "inogen" capable of rapid breakdown and rebuilding, if it is to be formed, as we have shown it must be, without the inclusion of oxygen. Yet the idea that the lactic acid, instead of being burnt away, may be actually restored into its former position in the molecule of its precursor, by the energy of a combustion of some other material in the oxidative recovery, has gained ground lately, and partly on account of an observation of our own. In our studies of the lactic acid of frog's muscle we made out the following facts. Suppose for a given set of similar muscles the "maximum" lactic acid production, as induced by heat rigor, be determined. Suppose, further, that another comparable set of muscles be stimulated to fatigue, then allowed to

recover in oxygen, again stimulated, and again submitted to oxygen, these alternate processes being many times repeated. It is clear that, since lactic acid is produced during each period of stimulation, and removed during each period of recovery in oxygen, heavy drafts must be made upon the precursor of the acid in any experiment such as that described. Nevertheless, a set of muscles, after having undergone such treatment, give, when thrown into heat rigor, exactly the same maximum yield of lactic acid as a set of perfectly fresh muscles (fig. 8).

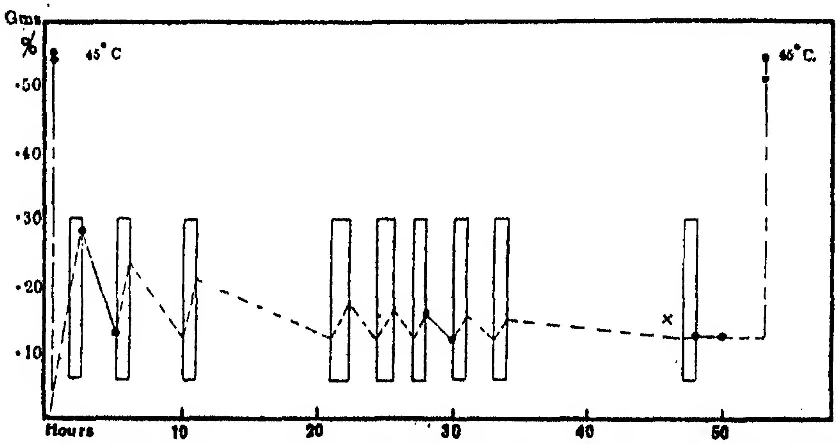


FIG. 8.—The relation of the heat-rigor lactic acid "maximum" to the survival history of muscle. Four estimations of lactic acid due to heat rigor are shown, two at the beginning, in the case of resting muscles, two at the 53rd hour, in the case of inexcitable muscles, which had gone through nine periods of severe stimulation alternated with periods of rest in an oxygen atmosphere. The enclosed areas represent time periods (drawn proportionate to abscissæ) of stimulation by strong interrupted shocks. × loss of excitability. Temperature 15° C. Continuous line shows course of acid loss as actually determined by estimation. Dotted line shows the presumed course of acid loss and gain during other alternate periods. (From the 'Journal of Physiology,' vol. 35, p. 293 (1907).)

In discussing these results, we suggested as a possible explanation (though we discussed alternative possibilities) that lactic acid is not oxidised during the recovery of the muscle, but is rebuilt into the complex from which it was derived, at the expense of energy derived from the oxidation of something else. The formation of this unstable complex would then be the basis for that restoration of potential which we have just been considering.

This view has been widely adopted, but we ourselves are now disposed to doubt it. It was shown at Frankfort by Kondo,* in Embden's laboratory, that the formation of lactic acid in expressed muscle-juice is due to a

* 'Biochemische Zeitschrift,' vol. 45, p. 63 (1912).

chemical reaction which is inhibited by acid, and is therefore self-controlled. It was suggested, therefore, that the maximum production in heat rigor does not represent the total exhaustion of the lactic acid precursor, but rather the normal cessation of a reaction at a critical concentration of hydrogen ions. So long as a sufficiency of the precursor is present, therefore, at the end of an experiment such as that described above, there is no difficulty about the attainment of a similar "maximum" by muscles restored after fatigue, and by fresh muscles, respectively. If this be so, our experiments gave no proof of a reconstruction of the lactic acid into something else. The experiments of Embden were not wholly satisfactory, because in muscle-juice lactic acid has so nearly reached a maximum, as the result of the expression of the juice, that the amount of change to be observed is but small. But, at our suggestion, Mr. Winfield has recently carried out some experiments at Cambridge upon intact muscles placed in Ringer's solution which fully confirm the fact that acid production in muscle depends upon a self-limiting reaction which ceases when a certain grade of acidity is reached. We are inclined, therefore, to doubt if there be any evidence on these direct lines that the lactic acid in recovery processes is rebuilt into a precursor in such a way as to restore the former level of the source of acid supply.

On this question of the possible restoration of lactic acid to its former molecular position, Hill has discussed some indirect evidence derived from his own thermodynamic studies. Peters found at Cambridge (14), by an adaptation of Hill's thermo-electric methods, that the heat production of chloroform rigor was equal to the sum of the two stages of heat production in similar muscles, in the first stage stimulated to give a partial lactic acid yield, and in the second stage killed with chloroform to give full rigor and the acid maximum. This observation in itself is interesting as an added proof that the heat production, like the lactic acid production, is derived from the same source whether in contraction or in rigor. Taking this total heat production found by Peters, together with our own lactic acid estimations for the same conditions, Hill argues that there is a heat production of 450 calories for each gramme of lactic acid formed. But his own results showed that the heat of oxidative removal of the acid was approximately equal to the heat of production, so that the oxidative removal of 1 grm. should yield about 450 calories. But the combustion of 1 grm. of lactic acid yields about 3700 calories. Hill urges accordingly that the indication of our experiment just quoted (fig. 8) should be accepted, and the lactic acid regarded as being replaced in its former position in the muscle during the oxidative recovery, the energy for that restoration of potential being derived from the combustion of some other constituent (*e.g.* carbohydrate)

in the muscle. The lactic acid on that view would be "part of the machinery and not part of the fuel," to use a familiar Cambridge phrase.

As opposed to this argument advanced by Hill we have, however, some more direct evidence supplied by Parnas, whose work at Cambridge with us was interrupted by the war, but has been continued in Germany (22). He compared the oxygen consumed by fresh resting and by fatigued muscles respectively, and thus determined the excess which was due to the process of recovery. From this he calculated the total heat which would correspond, in combustion, with the oxygen consumption observed. He then determined the actual heat production of similar muscles during the recovery process in oxygen after fatigue. This he found to be only half of that indicated by the oxygen consumption, and concluded that this retained energy was stored during the restoration of potential to the muscle. He suggests that the lactic acid is in part burned away, the heat supplying energy for restoring what he describes as the physico-chemical state of the resting muscle. Unfortunately, these experiments have not yet been described in detail, and it is therefore difficult to appraise their value. The technique used in the estimation of the heat given out by muscles is based upon the methods developed by A. V. Hill, and would appear to be satisfactory. One criticism occurs to us, however. The actual amount of energy presumed to be stored as potential in the fibres has relatively a very small caloric value: thus, during the whole process of complete recovery from full fatigue, Parnas found that only about two gramme-calories were stored per gramme of muscle. We are by no means clear that any correction was made for the latent heat of evaporation of water from the surface of the muscle, and this we conceive might greatly affect the quantitative value of such a measurement.

But it must be admitted that when fresh unfatigued muscles were used, there was apparently a close correspondence between the heat calculated from their oxygen consumption and that actually given out, which could hardly have been the case if surface evaporation had been occurring.

For the present we feel bound to conclude, upon the evidence as to heat production advanced both by Hill and by Parnas, that while the lactic acid produced during contraction is itself the material which is then immediately oxidised with a yield of carbon dioxide, part at least of the heat of combustion of lactic acid is stored in potential form in the muscle as it returns to the resting state.

We shall now endeavour to justify our belief that lactic acid itself is, as a matter of fact, the material actually oxidised in muscle.

Among those who, during recent years, have investigated, or considered with expert knowledge, the intermediary processes of metabolism, not from

the narrower standpoint of muscular activity alone, but in connection with the animal body as a whole, there has been almost complete unanimity in believing that lactic acid is an intermediary product on the main lines of carbohydrate metabolism. There is cogent evidence for this view, though it would, of course, be out of place for us to discuss it here. But it is the muscle in which by far the greater part of the total metabolism of the body takes place, and if, in muscle, lactic acid must be supposed in normal circumstances to appear only momentarily, and then, instead of following further steps towards the end-products of metabolism, to suffer instead a return to its source, it would be difficult to reconcile its history in muscle with what is believed concerning its importance in general metabolism.

It is significant in this connection to find, as we do (23), that the pancreas which exerts so important an influence upon the processes of general carbohydrate metabolism, exercises a direct control over the formation of lactic acid in muscle.

If it be not the lactic acid which is burnt, we must seek alternative fuel for the undoubted combustion which occurs. Hill suggested that carbohydrate as such was the fuel, giving energy for the restoration of the lactic acid to its former position. But Parnas and Wagner (24) have supplied definite evidence that carbohydrate, while it disappears from the muscle during the anaërobic processes in which lactic acid appears, remains unchanged in amount during the oxidative recovery.

Winfield at Cambridge has shown that fats, moreover, are not oxidised in the excised muscle (19), and we have good reasons, finally, to believe that in normal circumstances protein material is not burnt. But if neither protein, fat nor carbohydrate is the fuel we seek, what then is the material which undergoes oxidation? The only justification for doubting that it is lactic acid, the one substance which obviously accumulates in the absence of oxygen, and disappears in its presence, was the suggestion derived partly by tradition from the teaching of Hermann, and partly from the supposed evidence, already criticised, that the lactic acid disappears because from it is reconstituted the unstable substance, the "inogen," imagined to be the immediate source of the contractile energy. Apart from the absence of direct evidence in its favour, there are grave difficulties associated with the conception of an "inogen" capable of rapid breakdown and reconstruction, if we now abandon, as we have shown earlier that we must, the idea of the inclusion within it of oxygen. It seems almost impossible to conceive of an organic substance derived from lactic acid, and not containing "intramolecular" oxygen with unstable attachments, which could, by a non-oxidative rupture of its molecule, yield the energy required for contraction,

particularly if this, as Parnas's experiments seem to show, amounts to not less than half the oxidative energy of lactic acid. In our opinion the conception of a chemical "inogen" of any kind is false, and fated to disappear.

The high potential energy required for the rapid act of contraction may be stored, however, in some other form. The potential, which is lost upon contraction and restored by subsequent oxidation, may reside, not in an unstable chemical substance, but in a particular condition of a physico-chemical system. Here we may return to the image we previously depicted of the muscle machinery in our provisional hypothesis.

In a system of colloidal fibrils, or of longitudinal surfaces, into relation with which H-ions of lactic acid lie ready to be brought, we have a potential of energy which may be discharged as work, with or without heat, on the development of a new state of tension in the fibrils, whether tension due to inhibition or to added surface tension along the longitudinal surfaces. The observed heat production of anaërobic contraction may be in part due to the exothermic molecular change which yields the free acid from its precursor, and in part due to the resultant change in colloidal surfaces or substances upon the delivery to them of the acid ions.

Upon recovery by oxidative removal of the lactic acid, the energy of combustion is discharged in part as heat and in part (and what fractional part that is we have seen to be at present uncertain) returned to the muscle in the restoration of the initial potential. In this restoration will be involved the separation of the acid ions from the colloidal fibrils, by which the condition will be given for the return of the fibrils to their former tension—the tension, that is to say, of the muscle in the state of relaxation and rest, and possessed of the potential inherent in them.

We have been speaking so far of changes of potential in connection with the contractile act. As regards the actual main reservoir of energy, it is clear that this must be contained within the muscle itself, because most of our data have been obtained from excised muscle. That this main reservoir of energy is to be sought in the carbohydrate stores is, we believe, quite certain. The question arises, Are we to assume that carbohydrate must first be converted into a substance of higher chemical potential before it can serve as a contributory source of contractile energy by its breakdown to lactic acid? The small energy change which that breakdown involves has been thought by some to make this assumption necessary. It is just this assumption, however, that the conception of a change in the physico-chemical system of colloid fibrils, as the vehicle of a rise of potential, makes unnecessary. The contractile act may call, not only upon the chemical energy liberated when sugar becomes lactic acid, but also, and

perhaps to a greater degree, upon the energy derived from the oxidation of the lactic acid, residing in the physico-chemical system of the muscle, which was produced during the previous contraction.

If our picture of events is the true one, and if the machinery of contraction is of the kind we have suggested, then carbohydrate metabolism in muscle takes on an aspect of peculiar interest.

We have already recalled the evidence gained from studies in general metabolism, made without special reference to muscle, and have shown that it points clearly to the conclusion that sugar does not suffer oxidation as such, but only after it has first, at an early moment in its metabolic progress, passed through the stage of lactic acid.

But in the muscles, which after all form the chief seat of metabolism, the acid intermediary product appears, if we are right, at such a stage and place as to have more than a purely chemical significance. It marks, on the one hand, an obligatory stage in a particular set of successive chemical reactions; but, on the other hand, it has here its special *rôle* to play in connection with the muscle machinery. In the evolution of muscle it would appear that advantage, so to speak, has been taken of this acid phase in carbohydrate degradation, and that by appropriate arrangement of the cell elements the lactic acid, before it leaves the tissue in its final combustion, is assigned the particular position in which it can induce those tension changes upon which all the wonders of animal movement depend.

In concluding, we would endeavour to convey in brief terms our reasons for thinking that the particular standpoint thus taken is one which makes for simplicity and clearness in our views concerning muscle, and perhaps in more besides.

Underlying all views concerning the source of contractile energy, there has persisted till recently, almost as a tradition in physiology, the obstinate assumption that this energy must necessarily be sought in an unstable chemical substance of complex and unknown constitution—perhaps in the protoplasmic molecule itself, perhaps in an "inogen" vaguely to be distinguished from the protoplasm, perhaps only in some compound of a more definite sort in which carbohydrate matter finds itself transformed and endowed with a higher chemical potential. It must surely bring a gain to the clearness and simplicity of our conceptions, and bring encouragement also to the experimentalist, if such an assumption with its many attendant difficulties, to some of which we have alluded, should prove unnecessary. We believe it to be so. With an understanding that the relatively permanent physico-chemical system of the muscle can, without itself

undergoing chemical modification, carry changes of potential as a result of changes in its physical configuration, it becomes easier for us to realise that the food-stuffs, or at least that sugar, may be the direct source of the contractile energy. Placed in the right locality within the muscle, sugar, by a non-oxidative yield of acid at the right moment, and by a subsequent oxidation of this at another right moment, can yield its total energy in a manner exactly suited to serve the peculiar machinery in which, so to speak, it finds itself.

The actual chemical events which underlie the obvious manifestations of change in muscle—the contraction, the exhibition of fatigue, the recovery—we might then regard as relatively simple. We find similar indications in all progressive departments of biochemistry. The chemical events are not in themselves necessarily complex or obscure; the complexity is found in the conditions under which they occur. The difficulties of the biological enquirer arise from the fact that he has, for the most part, to accept these conditions as given. It is usually open to the physicist or pure chemist to control and simplify the conditions of his experimental work, or wisely to avoid regions of complexity until collateral progress has made them simple. In biology the complexities of the conditions are in the essence of the phenomena, and the experimentalist, when he tries to simplify them, is even viewed with suspicion. Thus even the operation of excising a muscle before studying its chemistry has been regarded with some prejudice, though in this case we think we may fairly claim that the progress made in the long series of enquiries we have discussed, has illustrated the fact that the biologist is after all not wholly shackled by the necessity of putting all his questions to the intact animal.

The description of muscle activity we have attempted to give remains, it is true, imperfect; indeed, we hardly yet have knowledge enough to guess how imperfect it is. But recent studies have had at least the result of confirming our own faith in the powers of experiment to bring improvement of knowledge, and we venture to believe that they have already indicated hopeful lines for further experimental work.

The following publications, to which reference has been made in the Lecture, are based upon researches which have been carried out in the Physiological Laboratory at Cambridge:—

(1) W. M. Fletcher, "The Survival Respiration of Muscle," 'Journal of Physiology,' vol. 23, p. 10 (1898).

(2) W. M. Fletcher, "The Influence of Oxygen upon the Survival Respiration of Muscle," *ibid.*, vol. 28, p. 354 (1902).

(3) W. M. Fletcher, "The Relation of Oxygen to the Survival Metabolism of Muscle," *ibid.*, vol. 28, p. 474 (1902).

(4) W. M. Fletcher, "The Osmotic Properties of Muscle and their Modifications in Fatigue and Rigor," *ibid.*, vol. 30, p. 414 (1904).

(5) W. M. Fletcher and F. G. Hopkins, "Lactic Acid in Amphibian Muscle," *ibid.*, vol. 35, p. 247 (1907).

(6) A. V. Hill, "The Heat produced in Contracture and Muscular Tone," *ibid.*, vol. 40 p. 389 (1910).

(7) A. V. Hill, "The Position occupied by the Production of Heat, in the Chain of Processes constituting a Muscular Contraction," *ibid.*, vol. 42, p. 1 (1911).

(8) W. M. Fletcher, "On the Alleged Formation of Lactic Acid in Muscle during Autolysis and in Post-survival Periods," *ibid.*, vol. 43, p. 286 (1911).

(9) F. Verzár (Budapest), "The Gaseous Metabolism of Striated Muscle in Warm-blooded Animals," *ibid.*, vol. 44, p. 243 (1912).

(10) A. V. Hill, "The Heat-production of Surviving Amphibian Muscles, during Rest, Activity, and Rigor," *ibid.*, vol. 44, p. 466 (1912).

(11) G. R. Mines, "On the Summation of Contractions," *ibid.*, vol. 46, p. 1 (1913).

(12) A. V. Hill, "The Energy degraded in the Recovery Processes of Stimulated Muscle," *ibid.*, vol. 46, p. 28 (1913).

(13) A. V. Hill, "The Absolute Mechanical Efficiency of the Contraction of an Isolated Muscle," *ibid.*, vol. 46, p. 435 (1913).

(14) R. A. Peters, "The Heat Production of Fatigue and its Relation to the Production of Lactic Acid in Amphibian Muscle," *ibid.*, vol. 47, p. 243 (1913).

(15) A. V. Hill, "The Heat Production in Prolonged Contractions of an Isolated Frog's Muscle," *ibid.*, vol. 47, p. 305 (1913).

(16) W. M. Fletcher, "Lactic Acid Formation, Survival Respiration and Rigor Mortis in Mammalian Muscle," *ibid.*, vol. 47, p. 361 (1913).

(17) W. M. Fletcher and G. M. Brown, "The Carbon Dioxide Production of Heat Rigor in Muscle and the Theory of Intra-molecular Oxygen," *ibid.*, vol. 48, p. 177 (1914).

(18) Viktor Weizsäcker (Heidelberg), "Myothermic Experiments in Salt Solutions in relation to the Various Stages of a Muscular Contraction," *ibid.*, vol. 48, p. 396 (1914).

(19) G. Winfield, "The Fate of Fatty Acids in the Survival Processes of Muscle," *ibid.*, vol. 49, p. 171 (1915).

(20) Parnas (Vienna), "The Transformation of Energy in Muscle," *ibid.*, vol. 49, p. vii (1914).

(21) Viktor Weizsäcker (Heidelberg), "Neue Versuche zur Theorie der Muskelmaschine," 'Münchener Med. Wochenschrift,' vol. 62, p. 217 (1915).

(22) Parnas (Vienna), "Ueber das Wesen der Muskelerholung," 'Zentralblatt für Physiologie,' vol. 30, p. 1, April, 1915.

(23) F. G. Hopkins and G. Winfield, "The Influence of Pancreatic Extracts on the Production of Lactic Acid in Surviving Muscles," 'Proc. Physiol. Soc.,' October 16, 1915.

(24) Parnas and Wagner (Vienna), "Ueber den Kohlenhydratumsatz isolierter Amphibienmuskeln und über die Beziehungen zwischen Kohlenhydratschwund und Milchsäurebildung im Muskel," 'Biochemische Zeitschrift,' vol. 41, p. 389 (1914).

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invisible, filterable stage, and that this also applies to laboratory cultures of organisms of certain diseases. In passing, I may note that my observations in 1914 in typhus fever, and, later, in the same disease,* in cerebrospinal fever, in scarlet fever and in measles, as to the existence in the infected body fluids of filterable infective viruses, and of growth from these of non-filterable bacteria, has since been confirmed in the case of laboratory cultures of the azotobacteria by Löhnis (2), in the summer of 1916. This observer, however, has recorded no details of his filtration experiments, and does not state if his cultures were from single colonies, or from single organisms, or if he carried out direct observations of growth from single individuals on the warm stage.

In the present communication I propose to present further pictorial evidence of the complicated life-history of the enteric group of bacteria in so far as this can be studied in laboratory media as opposed to the more natural *milieu* of infected tissues and body fluids. And to morphological studies of the members of the enteric group I have also added observations on a single strain of a coliform bacillus.

The strains of organism of the enteric group examined are as follows, their source being also indicated.

B. typhosus	4 strains: Strain 1, Lister Institute; Strains 2, 3, 4, Carrier strains, Addington.
B. paratyphosus A	3 strains: Strain 1, Lister Institute; Strains 2, 3, Carrier strains, Addington.
B. paratyphosus B	5 strains: Strain 1, Lister Institute; Strains 2, 3, 4, 5, Carrier strains, Addington.
B. Shiga-Kruse	1 strain: Lister Institute.
B. Y of His	5 strains: Strain 1, Lister Institute; Strains 2, 3, 4, 5, Carrier strains, Addington.
B. Flexner	3 strains: Strain 1, Lister Institute; Strains 2 and 3, Carrier strains, Addington.

In figs. 1–4 and Plates 16–19 will be seen the results obtained by study of dried film preparations from young cultures from single colonies, whilst in Plate 20, A, B, C, will be seen growth from single individuals studied on the warm stage.

All the strains of organisms shown were obtained from the Lister Institute, except the coliform organism.

It is not possible to reproduce here evidence that the morphological results noted in the case of all the strains enumerated above are identical with the

* Microphotographic evidence of growth from filterable virus to non-filterable bacteria in typhus fever, together with experimental evidence of pathogenicity at each stage, as well as evidence of complexity of life-history of the enteric organisms, was presented (3) to the Royal Microscopical Society in November, 1916.

results shown in the drawings and photographs of the selected strains. I must be content, therefore, with stating that the essential results were the same in all cases, approximately 1000 films having been examined. The organisms which I have chosen in order to illustrate my points here are the *B. typhosus* of Eberth, the *B. dysenteriæ* of Shiga-Kruse, the *B. dysenteriæ Y.* of Hiss, and a bacillus of the coliform group.

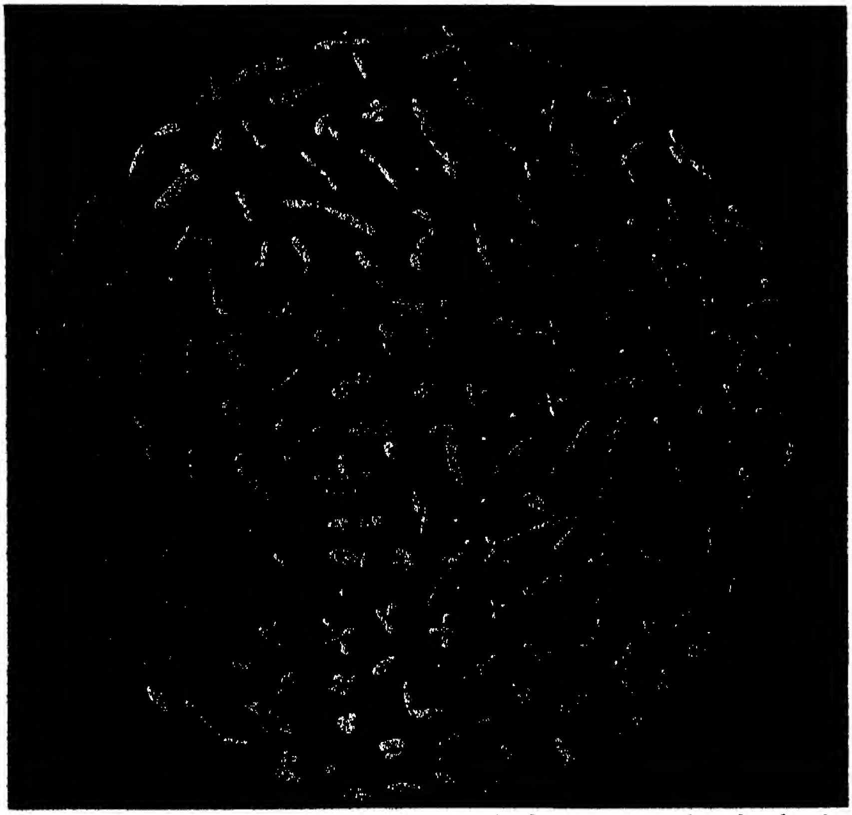

FIG. 1.—*B. typhosus*. +60 to phen. broth culture mixed with +10 to phen. broth culture from same.

In order to reduce the chances of error to the minimum I have, in addition to rigid precautions against contamination to be described later, submitted each strain of the organisms of the enteric group to searching identification tests, cultural, biochemical, and serological, both at the beginning, during the course of, and at the end of each set of observations, the additional precaution being taken of frequent replating on MacKonkey's medium and on agar, and of repeatedly restarting the whole process of examination by subculture from fresh single non-lactose-fermenting colonies on the former medium.

In every case the cultures under examination successfully passed at every stage the necessary standard identification tests, the final agglutination results of the three strains of members of the enteric group selected for demonstration being detailed below.

Shiga-Kruse Strain.—Final culture in broth, +10 phen. direct from broth, +20 phen. Dilution of antiserum 1/10, titre 1/1500, date of tubing 20.9.16 (Lister Institute).

Final dilutions ...	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560.	Control.
Result	Agg.	Agg.	Agg.	Agg.	Agg.	Agg.	Nil.	Nil.

Incubated for two hours at 56° C., and read after 24 hours at room temperature.

B. Typhosus Strain.—Final culture in broth, +10 phen. direct from broth, +60 phen.

Dilution of antiserum 1/20, titre 1/6000, date of tubing 31.3.16 (Lister Institute).

Final dilutions ...	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	Control.
Result	Agg.	Agg.	Agg.	Agg.	Agg.	Agg.	Agg.	Nil.

Incubated for two hours at 56° C., and read after one hour at room temperature.

B. "Y" of Hiss.—Final culture in broth, +10 phen. direct from broth, +20 phen.

Dilution of antiserum 1/10, titre 1/1500.

Final dilutions ...	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560.	Control.
Result	Agg.	Agg.	Agg.	Agg.	Agg.	Agg.	Nil.	Nil.

Incubated for two hours at 56° C., and read after 24 hours at room temperature.

The precautions taken against contamination are as follows, the culture media employed throughout being peptone agar and peptone broth, the latter +10 to phenolphthalein, +20 to phenolphthalein, and +60 to phenolphthalein, the last being only exceptionally employed.

1. The acid broth in each case was, after tubing, autoclaved for 30 minutes at 120° C. under a pressure of 20 lb.

2. Control tubes of uninoculated acid broth were incubated at 37° C. for the same length of time as the inoculated tubes. In no case was any turbidity or deposit observed after prolonged incubation.

3. In many of the cases the acid broth was contained in specially made silica glass flasks, the narrow necks of which were closed with rubber teats sterilised by one hour's immersion in pure lysol, and subsequently dried in sterile metal boxes for 24 hours at 56° C. It this way it was possible

entirely to avoid the use of plugs of wool, and to inoculate, or withdraw fluid from, the tubes with sterile Pasteur pipettes in the actual flame of a Bunsen burner. The use of these flasks also gave an absolute guarantee that only clean vessels were employed, each flask being heated to not less than 300° C. before use.

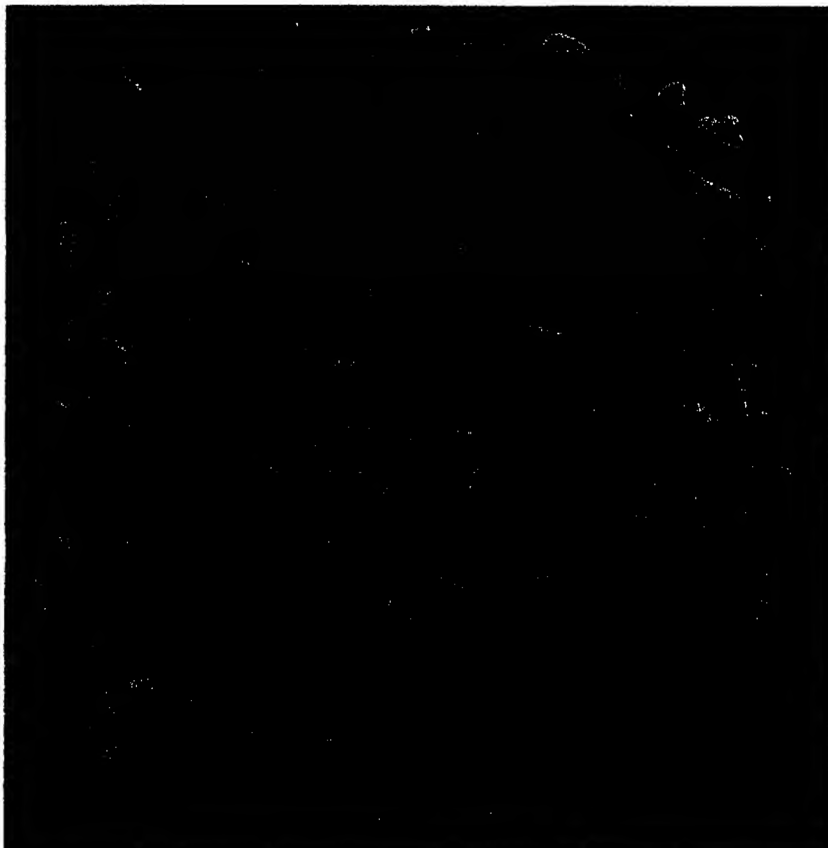

FIG. 2.—*B. coli communis.* Acid broth +20 to phen. mixed with broth sub-culture from same.

4. Deposits were obtained in all cases by centrifuging for three to five minutes the broth in small pointed serum tubes, each tube being sufficiently heated before filling to ensure carbonisation, and subsequent destruction of, any material left after routine cleaning by previous use. In this way it was possible to be certain that no organisms foreign to the inoculated broth under observation were present in the tubes employed.

5. All glass slides for microscopical examination were treated in the same

way, even after thorough cleansing with boiling acid and biohromate solutions. New slides were employed throughout.

6. The Congo-red emulsion* was made up daily, or on alternate days, with freshly distilled water, this being obtained for each set of experiments by distilling from a clean Jena flask fitted with new glass tubing. The

Equal Binary Fission.

A B

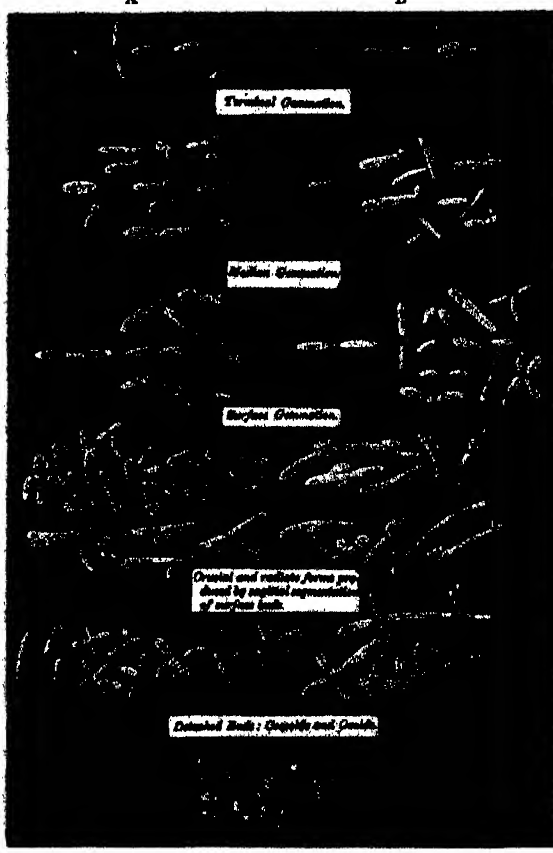

FIG. 3.—*B. Shiga-Kruse.* Acid broth +10 to phen. mixed with broth sub-culture from same.

emulsion was finally boiled in each case before use. In control films of Congo-red alone no organisms could be seen. In this way a dangerous source of error, due to mixing with tap water, or with distilled water from the ordinary laboratory still, was entirely avoided.

* In the case of dried films I employ a 1 per cent. solution of HCl in alcohol in order to avoid distortion of outline, control observations without the acid-alcohol bath showing that, if heat be not employed in drying, the use of a bath of this strength does not cause shrinkage or other distortion.

At the outset of the work, considerable difficulty was encountered in making satisfactory morphological studies of the organisms in question, on account of their small size, ordinary cultures of members of the enteric group providing organisms varying in size from approximately 0·5 μ to 2 μ. In Benians' Congo-red adsorption method, described by him in 1916 (4), I found, however, an invaluable method for studying the morphology of killed organisms, without any of the disadvantages inseparable from the use of basic stains, though in all cases the results obtained by the

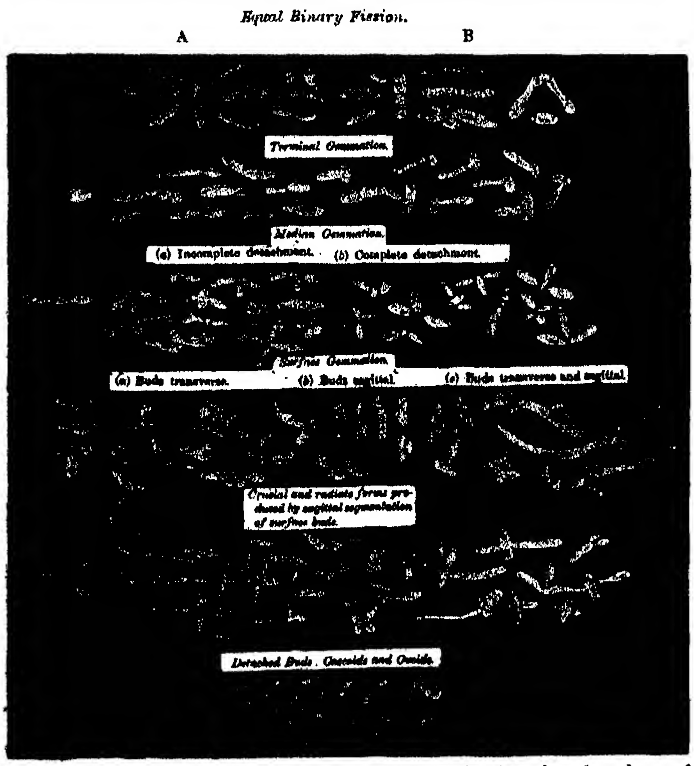

FIG. 4.—*B. Y of Hiss.* Acid broth + 20 to phen. mixed with broth sub-culture from same.

Congo-red method were confirmed by staining methods as well. The chief advantage of the method is the apparent increase in size of bacteria when emulsified with Congo red, as compared with the results obtained by staining methods, or even by Burri's adsorption method.

This apparent increase in size, however, was not sufficient for my purpose, as, although evidence of complex life-histories can—once one's attention has been arrested thereby—be unmistakably made out, both in stained films and in Congo-red films of ordinary cultures in + 10 broth, the size of the organisms

in these cultures is not sufficiently great to enable one to arrive with certainty at a correct interpretation of the different forms seen.

By the use, however, of broth +20 to phenolphthalein, and by sub-culture from this to agar or to MacKonkey's medium, thence back again to ordinary broth, +10 to phenolphthalein, I found that a great increase in size can with patience be obtained, still giving the classical cultural, fermentative, and serological reactions. This was particularly the case with the *B. typhosus*, with the *B. Shiga-Kruse*, with the *B. Y* of Hiss, and with the coliform organism examined. In the case of both the Paratyphoids, and of the *B. dysenteriæ* of Flexner, a considerable increase in size was also obtained by using these acid cultures, though so far I have not succeeded in obtaining the same increase as in the case of the other organisms mentioned.

Before going through the drawings, for which I am mainly responsible, and the photographs, for which Mr. Martin Duncan's skill and tireless enthusiasm are solely responsible, it is necessary briefly to deal with the possibility that many of the types of organism shown are merely involution forms.

That this is not the case is shown by the following considerations:—

1. The "aberrant" forms shown are young, freely growing, freely dividing organisms. By the use of the Congo-red method, the relative age of organisms can be fairly accurately gauged, owing to the fact that young organisms are brightly refringent, older organisms being faint or dark in colour.

2. In the case of the *B. typhosus*, the *B. Y* of Hiss, the *B.* of Shiga-Kruse, and the coliform organism, the growth in broth +20 to phenolphthalein was no less free and vigorous than in broth +10 to phenolphthalein, and it was in these that the largest and most "aberrant" types of organism occurred in the greatest numbers. Moreover, in broth +60 to phenolphthalein the degree of turbidity produced by the *B. typhosus* and by the organism of the coliform group was greatly in excess of that produced by these organisms in standard broth, +10 to phenolphthalein.

3. These "aberrant" types maintained their "aberrancy" for several sub-cultures when put back into broth +10 to phenolphthalein, even when the latter was inoculated direct with single colonies from MacKonkey's medium.

4. They were seen in small numbers, if carefully searched for, in ordinary cultures in broth, +10 to phenolphthalein, which had never been inoculated from broth of a higher acid titre.

This was also true of single colonies on MacKonkey's medium, or on agar, that had not at any time been derived from incubated broth cultures, but had been isolated direct from the fæces or urine of carriers.

5. The same types of "aberrancy" were seen in every one of the different organisms shown, as well as in all the strains of all the organisms not shown, and of the different strains of the organisms, single strains of which are shown.

In the accompanying photographs (Plates 16–19) and drawings attention is called to the following points :—

1. It is not claimed that a complete history of bacterial life-cycles can be worked out in acid broth cultures. This can only be obtained by extensive experimental observations, side by side with comprehensive morphological studies of organisms as they occur in the infected tissues and body fluids of subjects of disease. These, in the case of members of the enteric group, have yet to be undertaken.

2. In all the broth cultures studied, reproduction by simple binary fission was still the predominant feature, and in studying the "aberrant" types of reproduction of single living organisms on the warm stage on solid media, such as gelatin-agar, ordinary binary transverse fission was found eventually to hold the field mainly, though not absolutely, to the exclusion of other types of reproduction. It appears from these observations from single living individuals on the warm stage that reproduction by gemmation occurs freely, in conjunction with ordinary binary fission, only so long as growth proceeds in the thin layer of broth on the cover-slip, and largely comes to an end when colonies are beginning to form on the solid medium. This abrupt transition is well seen in Plate 20, as is also the familiar "slipping" phenomenon described by Hill in 1904.

3. The percentage population of "aberrant" forms in ordinary broth cultures, +10 to phenolphthalein, was low, but the chief types of "aberrancy" recorded could always be found if persistently searched for.

4. The percentage population of "aberrant" forms in broth cultures +20 and +60 to phenolphthalein, as well as in sub-cultures of these in ordinary broth cultures, +10 to phenolphthalein, was high, each field of the microscope yielding, in good films, characteristic types.

5. Each photograph is designed to show at the optimum focus not more than a very small number of types.

6. The number of types which can be seen in any acid culture exceeded 100. To reproduce a comprehensive picture by photographs alone was therefore impracticable.

7. In consequence it became necessary to make composite drawings with the camera lucida of the chief types observed in one or, at the most, two film preparations from one strain. These drawings represent, in the case of killed organisms, selected individuals from a large number of fields, and must therefore not be read as representing average fields.

8. At first sight inspection of the drawings in figs. 1 and 2, and of the photographs of killed organisms, suggests meaningless chaos.

9. Once, however, it is grasped that reproduction by gemmation is the key to the "aberrant" forms shown, and that gemmation may be terminal, median or superficial, the main types fall into line.

That true gemmation occurs of these three types is shown in Plate 20, representing growth from single organisms on the warm stage.

10. There is no evidence that a given strain represents a mixture of several strains, this suggestion being largely excluded by study of gemmating forms before fission has taken place, and by study of the actual process of gemmation on the warm stage.

11. The correct explanation of the superficial gemmation origin of the crucial and radiate forms shown is more difficult to establish than is that of the terminal, median and simple superficial forms of gemmation.

The points against a mere apposition explanation of these crucial and radiate forms are as follows:—

(a) Strict rectangular symmetry is the rule.

(b) The diameter of the central, brightly refringent node is frequently twice that of the organism which might otherwise be interpreted as lying in contiguity.

(c) Superficial buds can frequently be observed on the parent bacillary stem before "sprouting" has commenced, and during the act of sprouting.

12. Sagittal segmentation of buds can frequently be seen, both in the case of dried organisms and of single living organisms, before separation from the parent stem has begun. This sagittal segmentation can be seen in the terminal, median, and superficial buds.

13. Transverse segmentation of buds—ordinary binary fission—also frequently occurs, the parent stem also presenting buds undergoing sagittal segmentation, the actual occurrence of which was watched on the warm stage, as shown in Plate 20.

14. Undetached buds may vary in size from about $0 \cdot 1\,\mu$ to several μ in their greater diameter, every intermediate size—from the filterable to the non-filterable—being capable of recognition in the same film in favourable cases (*vide* photographs of dried films).

15. The appearance of minute buds on a large scale is inconstant in broth cultures, as observed in dried films. In the study of growth from single organisms on the warm stage it occurs frequently, only a relatively small

number, however, coming to maturity on solid media, the majority fading and disappearing.

16. The appearance of the very minute forms, seen in figs. 1, 3, and 4, and Plates 16 and 20, makes it impossible to be certain, without prolonged observation on the warm stage, that, in attempting to obtain cultures from single individuals of normal size by Barber's method, or by the fragmented slip method of isolation, one is not in reality cultivating from several individuals. Unless therefore the presence of these minute forms can be excluded, the use of these two methods for obtaining cultures in liquid media from single organisms cannot be relied on.

17. The presence of these minute forms is probably the explanation of the apparent filterability through Chamberland filters of such relatively large organisms as the *Bacillus bronchisepticus*, and is perhaps responsible for the general view that even well-made Berkefeld filters are not suitable for bacteriological work.

18. By the dark-ground method of illumination many of these small detached buds appear as minute bacilli in the act of undergoing binary fission. Not infrequently these appear as coccoid bodies, if binary fission has not begun. In the study of aberrant bacterial forms with dark-ground illumination the use of the hanging drop method, apart from the inherent fallacies of dark-ground work, is fatal to correct interpretation unless streaming movements have first been reduced to a minimum. For example, it is frequently stated that apparent branching in bacteria is, in reality, simulated by mere apposition, and that observation of a hanging drop with dark-ground illumination will soon dispel the illusion, separation of apposed organisms sooner or later always taking place. On casual inspection of dark-ground hanging drops this statement appears to represent the truth, especially if streaming movements are still free. If, however, a drop of emulsion be firmly pressed under a cover-slip and then examined, it will be found, streaming movements now being reduced to a minimum, that detachment does not invariably take place. That this is not the result of pressure is shown by the fact that in favourable cases long lateral buds will exhibit wide lateral movements, whilst the base, or point of attachment to the parent stem, remains fixed. In other cases short lateral buds retain their relative position to the parent stem, itself exhibiting unfettered rotatory movements. The accuracy of these observations is confirmed in Plate 20 of warm stage studies.

In concluding it is perhaps unnecessary to point out that no claim whatever is here made that the complete life-histories of the bacteria of the

enteric group have been worked out. On the contrary the sole aim has been to show that simple binary fission is not the only method of reproduction of these organisms, and that only a fraction of what appears to be a highly complex life-cycle can be studied by cultivation in, or on, synthetic media.

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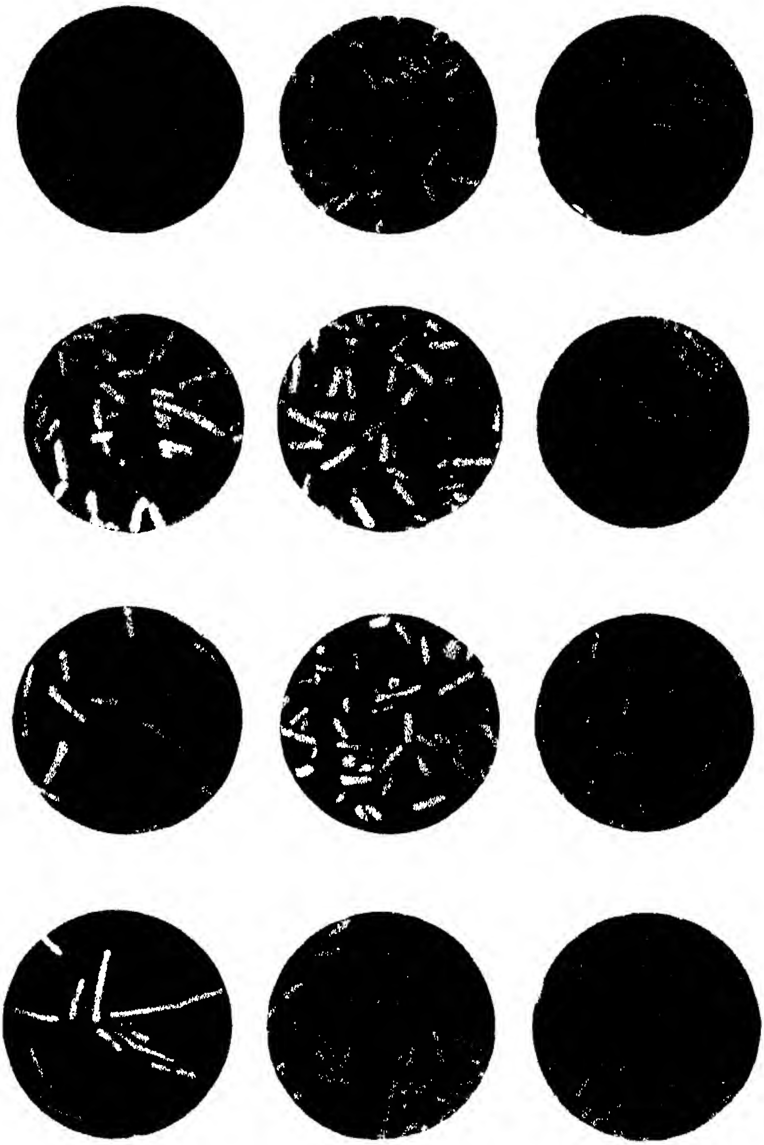
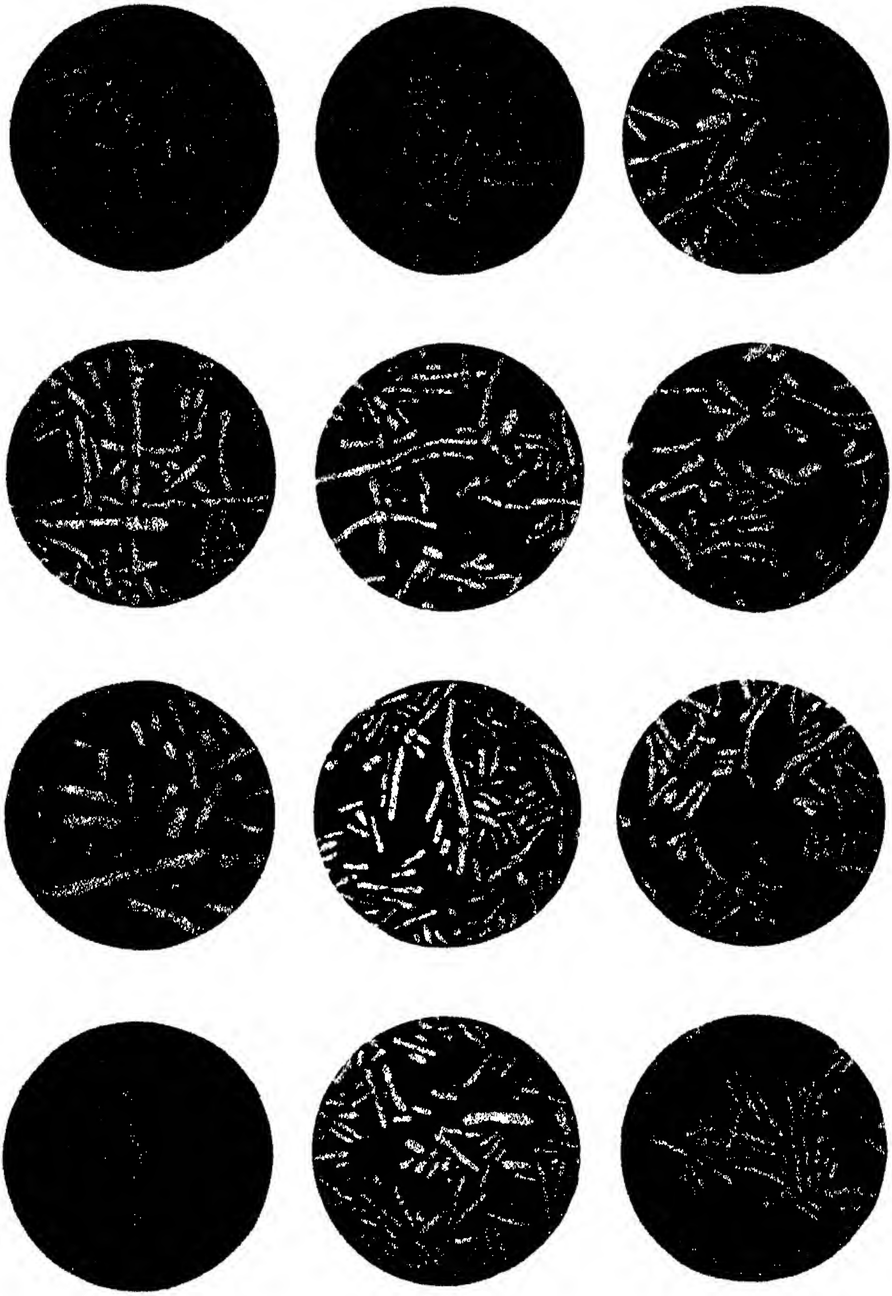
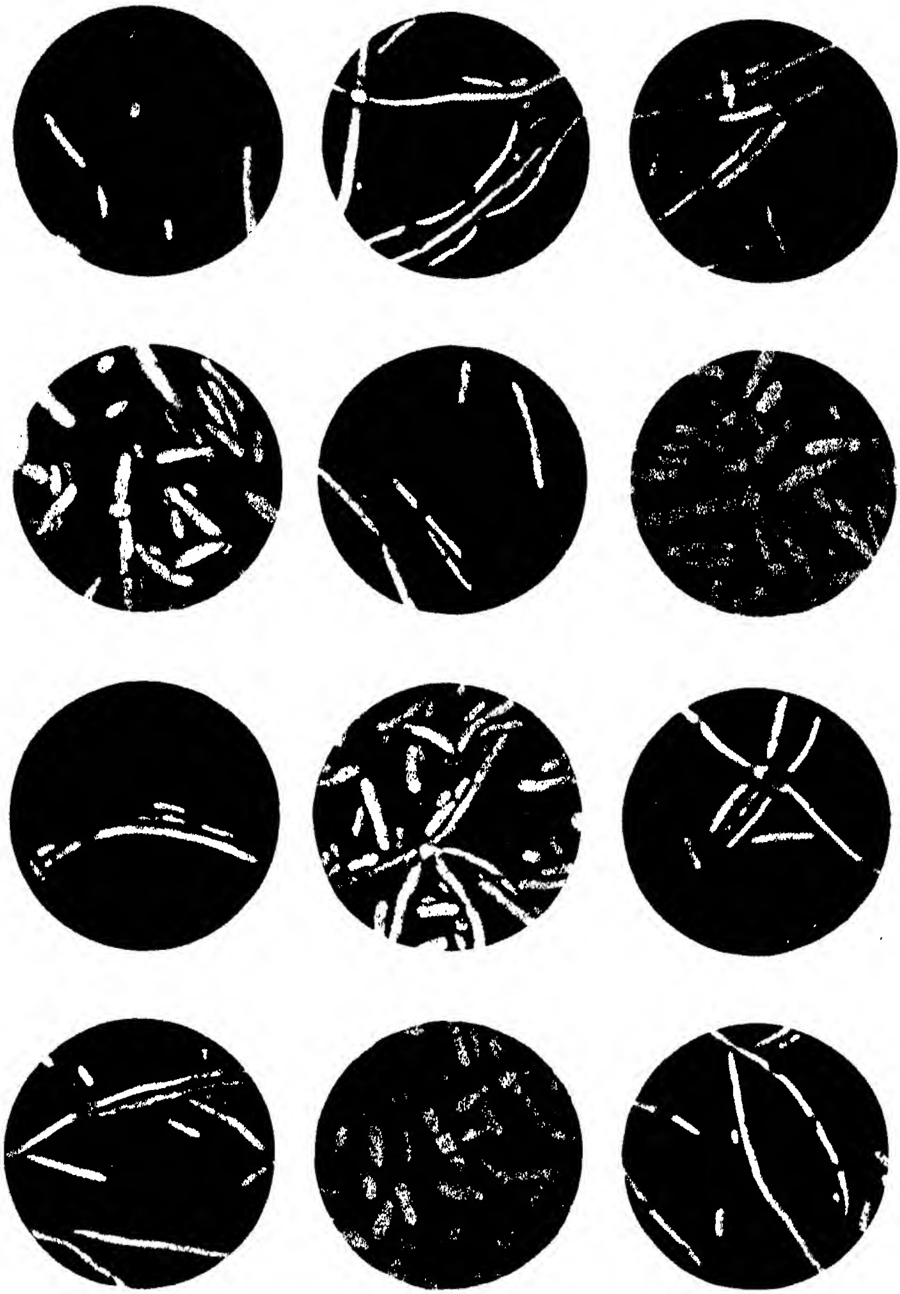


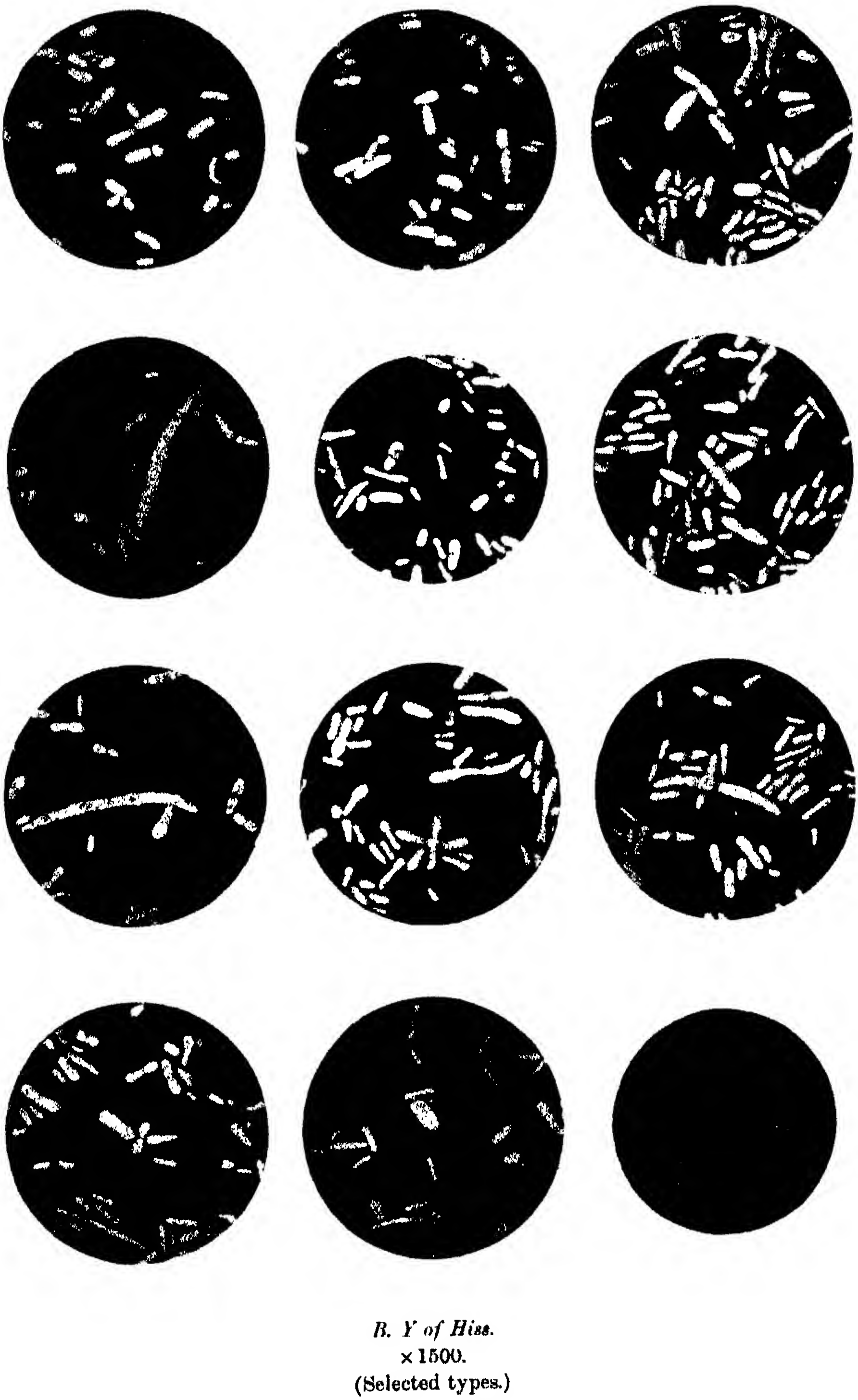
B. Shiga-Kruse.
× 1500.
(Selected types.)



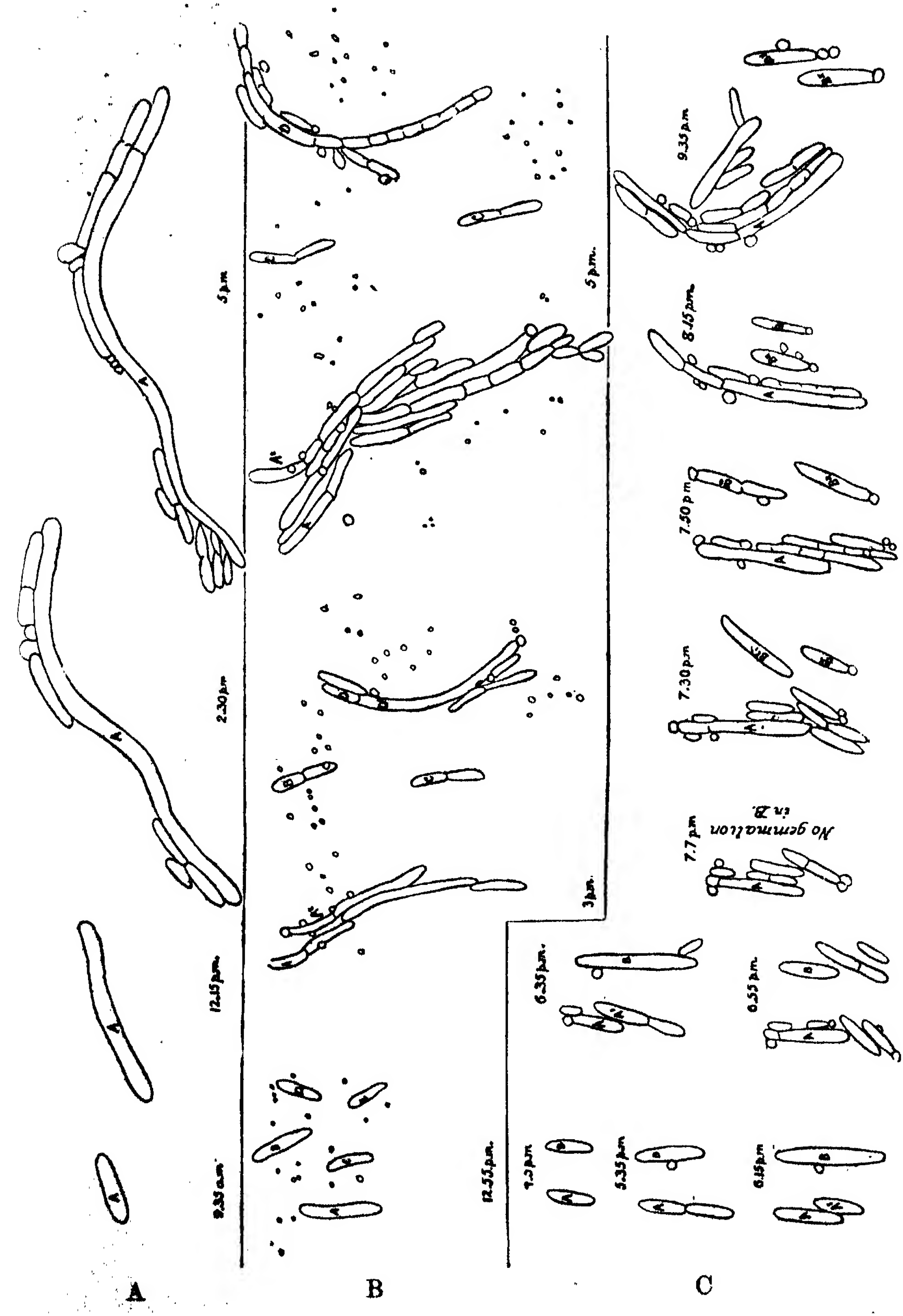
B. Coli Communis.
×1500.
(Selected types.)



B. Shiga-Kruse.
× 1500.
(Selected types.)



B. Y of Hiss.
×1500.
(Selected types.)



5 p.m.
2.30 p.m.
12.15 p.m.
9.35 a.m.
5 p.m.
3 p.m.
12.55 p.m.
9.35 p.m.
8.15 p.m.
7.50 p.m.
7.30 p.m.
7.7 p.m.
No germination in B.
6.35 p.m.
6.55 p.m.
9.5 p.m.
5.35 p.m.
6.15 p.m.
A
B
C

Some Effects of Organic Growth-Promoting Substances (Auximones) on the Growth of Lemna minor *in Mineral Culture Solutions.*

By W. B. Bottomley, M.A., Professor of Botany, University of London, King's College.

(Communicated by F. W. Oliver, F.R.S. Received January 2, 1917.)

[Plates 21 and 22.]

Introduction.

In a previous communication* experiments were described which indicated that when peat is incubated with a mixed culture of aërobic soil organisms for about fourteen days at a temperature of 26° C., a rapid decomposition of the organic matter takes place, with the formation in the "bacterised" peat of certain organic growth-promoting substances or "auximones,"† the addition of which in very small amounts to wheat seedlings growing in water culture causes a marked increase in growth. It was desirable to repeat these experiments with plants in which any variation in growth could be readily and more accurately estimated than in wheat seedlings.

There was a difficulty at first in selecting a suitable plant for experiment. The objections to using the seedlings of land plants are: the difficulty of accurate weighings at regular intervals; the fact that a water culture solution is not the natural habitat for a land plant; the possibility that such seedlings may contain a supply of organic growth-promoting substances produced from the endosperm during germination. Water plants, on the other hand, are usually considered unsuitable for water culture experiments because they will not grow for any length of time in pure mineral culture solutions. Darwin and Acton‡ state that "water plants cannot generally be recommended for accurate experiments extending over any considerable time, as we have found it much more difficult to grow them satisfactorily in culture solutions than to grow ordinary plants with the roots immersed." They say, however, "we have found *Lemna minor* useful for purposes of

* 'Roy. Soc. Proc.,' B, vol. 88, pp. 237–247 (1914).

† In a previous communication ('Roy. Soc. Proc.,' B, vol. 89, p. 102 (1915)) the term "auximone" (Gr. αὔξιμος: promoting growth) has been suggested for these substances. This term may usefully serve as a general descriptive name for these organic plant growth-promoting substances until our knowledge of their true nature and composition is sufficiently extended to warrant the application of a more satisfactory name.

‡ Darwin and Acton, 'Practical Physiology of Plants,' pp. 61–63 (1901).

demonstration. They grow rapidly, and their increase being principally in one plane is easily noticed at a glance. Moreover a rough numerical estimate of the amount of increase in a given time can be made by counting the fronds."

A preliminary experiment with *Lemna minor* growing in water culture solutions showed that this plant responds readily to the presence of auximones, and, when supplied with these substances, there is strong and healthy growth for an indefinite period. Also by counting the number and measuring the areas of the fronds at regular intervals of time when growing in different culture solutions, a reliable estimate of variations of growth can be obtained.

Accordingly a detailed investigation of the influence of certain auximones on the growth of *Lemna minor* was commenced. On July 1, 1915, three similar porcelain dishes of 1 litre capacity were prepared: one containing 600 c.c. of Detmer's complete culture solution made up with glass-distilled water; the second, the same amount of Detmer's solution with the addition of the decomposed phosphotungstic acid fraction of bacterised peat, extracted as described in the previous communication, in the proportion of 17 parts of dry substance per million of solution; and the third, Detmer's solution plus the silver-baryta fraction of bacterised peat, in the proportion of 0·35 part per million.

Thirty healthy plants of *Lemna minor* of uniform size were counted out into each of the three dishes. The dishes were then covered with glass plates to exclude dust, and were kept in a cool greenhouse. At the end of a week the number of plants in each dish was again counted, their area measured by means of squared paper, and the liquids replaced by fresh solutions. This treatment was repeated weekly, with slight irregularities, until October 20.

At the end of six weeks the plants receiving the phosphotungstic fraction had filled their dish, therefore the contents of the three dishes were divided exactly into half at the weekly counting, and one-half of each set was discarded. This had to be repeated frequently owing to the very rapid multiplication of the auximone plants, until on October 20, when the experiment was concluded, the number of plants in the respective dishes had then to be multiplied by 256, to obtain the total numbers which would have been derived from the original 30.

The final figures obtained showed a percentage increase in number over the Detmer's solution of 197 for the phosphotungstic fraction, and 109 for the silver fraction.

The plants receiving auximones retained their vigour and maintained

their original size of 3·73 sq. mm. throughout the experiment, whilst the control plants soon began to show signs of starvation, and decreased in size week by week, until at the end of the experiment they had diminished from 3·73 to 0·78 sq. mm., and it became difficult to count and measure them accurately.

Microscopic investigation also showed striking differences in internal structure. In the control plants there was an excessive number of large air spaces. The individual cells were small, with a large central vacuole and a small nucleus. In the auximone plants the tissues were more compact, with fewer and smaller air spaces. The large cells were filled with cytoplasm and possessed a well-developed nucleus which stained deeply.

In these experiments there was only one dish of plants for each variation of culture solution, and it was considered that "the result could not be regarded as conclusive, owing to the probability of experimental errors." It was therefore necessary to repeat and extend the experiments with a larger number of dishes for each culture solution. After consultation with certain botanical colleagues, it was decided that each set should consist of 10 dishes, that the rate of growth should be estimated by (1) increase in number of plants, (2) increase of dry weight, instead of measurement, each time the sets were halved, and that other extracts of bacterised peat should be tested.

Owing to the lack of suitable greenhouse accommodation at King's College for these experiments, the authorities of the Botanical Department of the Imperial College of Science and Technology, South Kensington, kindly granted permission and offered facilities for the work to be carried out in the greenhouse laboratory of that college.

Experiments with Ordinary Distilled Water.

On June 9 last year, five series, each consisting of 10 dishes, were prepared, and were numbered from 1 to 50. Flat-bottomed glass crystallising dishes of 4 inches diameter were employed, all containing 250 c.c. of the required solution. Each of the dishes was then enveloped on the outside, to the level of the contained liquid, with paper which was dull black on one side and white on the other, the black side being towards the dish and the white towards the exterior, in order to prevent as much as possible access of any heat and light rays through the sides and bottom of the vessels.

The solutions employed were:—Series I, Detmer's standard culture solution; Series II, Detmer's solution, together with a water extract of bacterised peat; Series III, Detmer's solution, with a similar extract freed from humic acid; Series IV, Detmer's solution, plus an alcoholic extract of

bacterised peat; Series V, Detmer's solution, with the addition of the phosphotungstic acid fraction of bacterised peat. No experiments were made with the silver fraction on account of the lack of available material at the commencement of the experiment.

Detmer's solution was the standard solution consisting of potassium nitrate, 7 grm.; dipotassium phosphate, 1·5 grm.; magnesium sulphate, 1·5 grm.; sodium chloride, 1·5 grm.; calcium sulphate in excess (5 grm. were used); ferric chloride solution, a few drops; and distilled water, 3000 c.c. This solution contained nitrogen, phosphorus, and potash, estimated as NH_3, P_2O_5, and K_2O, in the proportion of 393, 204, and 1220 parts per million respectively, the total concentration of salts being about 5500 parts per million.

The water extract of bacterised peat was prepared by leaching out, by means of boiling distilled water, all soluble matter from a weighed quantity of bacterised peat, and making up the liquid to a known volume. An aliquot portion of this solution, containing the extract from ½ grm. bacterised peat, estimated on the dry weight, was added to each of the dishes containing Detmer's solution in Series II.

The water extract of bacterised peat, freed from humic acid, was prepared by precipitating the humic acid from a water extract prepared as above, by the requisite amount of a very dilute solution of calcium chloride, and removing the calcium humate by filtration. An aliquot portion of this liquid, representing the extract from ½ grm. peat, was added to each of the dishes in Series III.

The alcoholic extract and the phosphotungstic fraction were obtained by the methods described in the previous communication. The alcoholic extract from 1 grm. of bacterised peat was added to each of the dishes in Series IV, and the phosphotungstic fraction from 2½ grm. to each dish in Series V.

In order to ensure the use of uniform culture solutions, stock solutions of all the required substances, sufficient to last throughout the whole of the experiment, were prepared at the outset. The Detmer's culture solution was prepared in a concentration of 100 times the strength required, and the various organic extracts were prepared in concentrated solutions, to which a little chloroform water was added to prevent bacterial action. The chloroform was removed by gentle evaporation on the water-bath each time the liquids were required for use.

The proportions of total substances added in the various series to the 5500 parts per million of mineral salts in the Detmer's solution are shown in the Table below.

Table I.

Series.	Total solids added (parts per million).	Organic substance (parts per million).	Inorganic matter (parts per million).
II	421	368	53
III	189	97	92
IV	44	32	12
V	17	13	4

The greater quantity of inorganic matter in Series III than in Series II is explained by the substitution of the inorganic chloride for the organic humate radicle, and possibly also by a slight excess of calcium chloride used.

An estimation of the mineral nutrients, nitrogen, phosphorus and potash, was made only for Series II, as this series contained the maximum addition of these nutrients. The figures obtained for these additions, as compared with the quantities already present in Detmer's solution, are as follows:—

Table II.

	Amount of nutrient present in Detmer (parts per million).	Amount of same nutrient added (parts per million).	Percentage of addition.
Nitrogen (estimated as NH_3)	393	19·2	4·8
Phosphorus (estimated as P_2O_5) ...	204	3·6	1·8
Potassium (estimated as K_2O) ...	1220	0·52	0·04

Twenty plants of *Lemna minor,* as nearly uniform as possible in size, general healthiness and root development, were counted out into each of the 50 dishes. The solutions were changed twice each week in order to maintain, as nearly as possible, the original balance of the salts, and to eliminate bacterial contamination; and, at each changing, the dishes were thoroughly cleansed before the solutions were renewed. The plants in each dish were counted every week.

The plants in all the series multiplied fairly uniformly for the 1st week, then the effect of the auximones in Series II and III became very marked, until at the end of three weeks the plants in these series completely filled their dishes. At this stage the plants in all 50 dishes were halved, one half being retained, and the dry weight of the other half estimated. This was repeated for a further three weeks, but at the end of the 7th week the plants in Series II and III had become so numerous that they had to be quartered, one-quarter only being retained instead of one-half.

Table III.

Series No.	Set No.	Beginning of expt.	1st week.	2nd week.	3rd week.		4th week.		5th week.		6th week.		7th week.	8th week.
		No. of plants.	No.	No.	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.	No.
						mgrm.		mgrm.		mgrm.		mgrm.		
I	1	20	31	49	82	10·0	148	10·4	208	8·0	336	12·8	608	1,120
	2	20	30	51	74	8·4	140	8·8	184	8·0	304	12·8	544	1,152
	3	20	31	52	78	11·6	128	12·8	192	14·4	304	19·2	576	1,056
	4	20	26	44	68	10·0	112	12·0	168	12·8	288	19·2	416	768
	5	20	30	55	74	8·8	140	9·6	248	11·2	352	19·2	640	1,120
	6	20	31	55	88	11·2	144	14·4	232	16·0	400	22·4	640	1,312
	7	20	31	56	72	9·6	148	8·0	200	14·4	288	19·2	416	896
	8	20	34	56	82	11·2	140	16·0	264	12·8	336	16·0	476	1,056
	9	20	31	57	84	10·8	140	14·4	240	14·4	352	22·4	608	1,184
	10	20	28	47	64	9·2	112	12·8	176	12·8	304	12·8	480	864
	Mean	20	30·3	52·2	76·6	10·1	135·2	11·9	211·2	12·5	326·4	17·6	550·4	1,052·8
II	11	20	41	98	326	55·2	1,104	185·6	3,016	483·2	7,312	1,161·6	20,032	77,312
	12	20	36	97	324	47·6	1,128	192·0	3,272	515·2	7,056	1,155·2	19,904	74,496
	13	20	36	108	340	54·0	1,108	117·6	3,064	491·2	6,144	1,049·6	20,096	57,856
	14	20	37	100	290	45·2	980	169·6	2,728	484·8	6,032	976·0	18,880	68,352
	15	20	37	96	292	56·4	920	133·6	2,568	441·6	5,712	918·4	18,624	62,464
	16	20	33	98	302	54·4	1,032	188·0	2,960	529·6	7,040	1,206·4	19,328	74,752
	17	20	40	111	382	63·6	1,300	208·8	3,408	587·2	7,888	1,283·2	20,800	62,208
	18	20	37	110	366	58·0	1,308	219·2	3,696	580·8	7,312	1,139·2	21,696	75,776
	19	20	39	101	320	53·6	1,044	162·4	2,816	436·8	6,128	1,056·0	17,216	65,024
	20	20	39	107	322	48·8	1,080	174·4	3,120	566·4	6,608	1,084·8	21,056	75,264
	Mean	20	37·5	102·1	326·4	53·7	1,100·4	181·1	3,064·8	511·7	6,723·2	1,103·0	19,763·2	69,350·4

III	21	20	36	77	218	30·0	576	87·2	1,320	244·8	3,120	492·8	9,472	35,456
	22	20	38	77	206	28·4	568	84·0	1,360	193·6	2,992	505·6	9,408	38,272
	23	20	35	76	176	22·8	448	58·4	1,232	177·6	2,768	448·0	8,512	35,968
	24	20	37	80	204	28·8	528	77·6	1,392	203·2	2,944	499·2	9,856	38,144
	25	20	38	82	202	30·8	544	86·4	1,464	222·4	3,104	553·6	10,560	42,624
	26	20	32	74	214	31·6	616	92·8	1,544	240·0	3,504	588·8	10,688	39,936
	27	20	34	70	188	28·0	476	68·8	1,216	172·8	2,944	441·6	8,576	38,912
	28	20	36	81	228	37·6	788	122·4	1,664	238·4	3,760	652·8	10,112	41,856
	29	20	35	70	210	28·4	608	82·4	1,544	256·0	3,536	582·4	11,776	42,368
	30	20	34	72	164	22·4	460	63·2	1,216	187·2	2,576	400·0	8,704	34,304
	Mean	20	35·5	75·9	210·0	28·9	561·2	82·3	1,395·2	213·6	3,124·8	516·5	9,766·4	38,784
IV	31	20	35	63	124	20·4	256	26·4	504	65·6	1,040	108·8	3,040	12,608
	32	20	34	55	110	14·0	304	23·2	584	65·6	1,136	137·6	3,136	14,336
	33	20	33	61	132	18·4	304	34·4	576	62·4	1,216	140·8	3,264	14,060
	34	20	32	60	114	14·8	284	24·8	488	44·8	992	105·6	2,848	11,776
	35	20	36	63	132	17·2	312	37·6	656	78·4	1,328	166·4	4,544	16,064
	36	20	35	66	142	21·2	304	42·4	680	83·2	1,504	204·8	5,184	15,744
	37	20	31	53	104	13·2	224	21·6	464	44·8	912	102·4	2,336	10,496
	38	20	33	57	122	14·0	252	28·8	432	46·4	800	99·2	2,144	10,752
	39	20	35	60	126	17·6	288	33·6	528	59·2	1,024	118·4	2,880	12,416
	40	20	29	58	112	16·0	276	31·2	512	64·0	1,056	112·0	2,592	11,520
	Mean	20	33·3	59·6	121·8	16·7	280·4	30·4	542·4	61·4	1,100·8	129·6	3,196·8	12,979·2
V	41	20	37	62	96	12·4	184	20·8	312	24·0	512	54·4	864	1,984
	42	20	34	52	90	12·8	152	14·4	272	27·2	448	44·8	928	2,016
	43	20	35	56	96	12·0	168	18·4	256	24·0	416	38·4	832	1,504
	44	20	33	62	96	11·6	176	20·8	272	25·6	448	41·6	832	1,920
	45	20	30	55	86	10·0	168	19·2	280	30·4	464	48·0	1,024	2,656
	46	20	34	61	112	15·6	200	24·8	328	32·0	592	51·2	1,376	3,104
	47	20	32	55	92	12·8	176	23·2	296	27·2	528	38·4	1,056	2,496
	48	20	29	50	88	12·4	152	17·6	272	30·4	448	48·0	960	2,016
	49	20	33	55	92	11·6	168	18·4	320	28·8	512	44·8	992	2,240
	50	20	29	48	84	11·6	160	17·6	296	28·8	464	48·0	896	3,752
	Mean	20	32·6	55·6	93·0	12·3	170·4	19·5	290·4	27·8	483·2	45·8	976·0	2,268·8

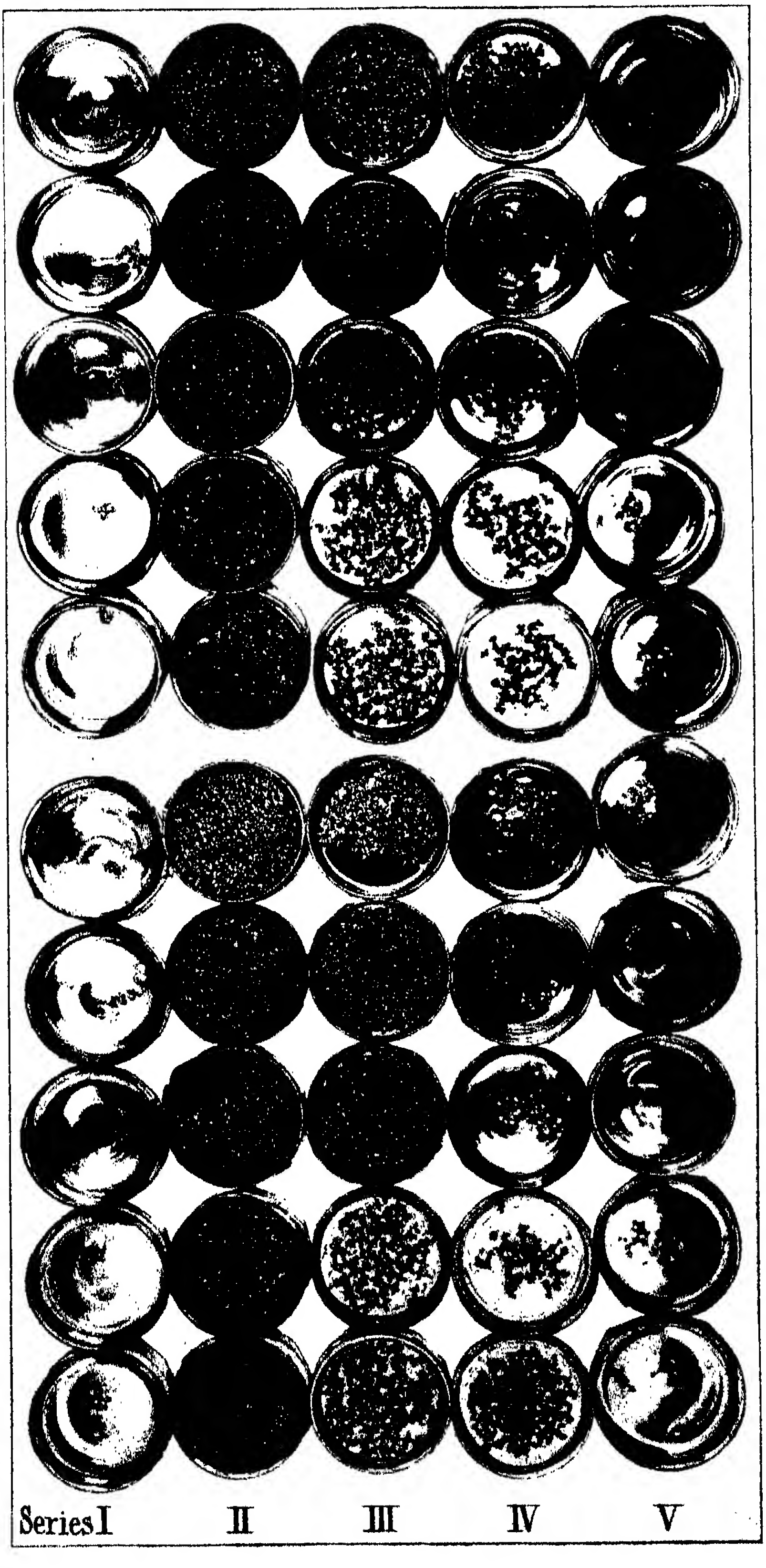
The total number of plants each week, and their dry weight for the 3rd and succeeding weeks, are shown in the Table on pp. 486–7, the numbers representing, after the 2nd week, not those which were actually in the dishes, but the numbers which would have resulted from the multiplication of the original 20 had the dishes used been sufficiently large to obviate the need for halving and quartering. The dry weights also correspond to the weights of the complete sets whose numbers are given, and not to the fractions of the whole sets which were weighed each week. By the end of the 6th week, the labour involved in counting, halving, and estimating the dry weight of the plants in the 50 dishes had become so great that it was impossible to continue the whole of the work further. The experiment was carried on, however, for another two weeks, with the object of recording only the numbers of the plants in the various series.

It is evident from the above figures that all the additions to Detmer's solution have a beneficial effect on the growth of the *Lemna minor* in water culture, and that there is a progressive beneficial effect both in number and weight as the amount of added substances is increased. The most striking results are shown in Series II with the complete extract of bacterised peat, which in six weeks increased the numbers by 20 times (6723 : 326), and the weights by 62 times (1103 : 17·6), those in Detmer's solution. As the additions made consisted chiefly of organic substances, the conclusion appears to be justified that it is the presence of organic matter which is responsible for the results obtained, for it is difficult to understand how such increases could have been brought about by the addition of inorganic nutrients only, since the maximum proportions of nitrogen, phosphorus and potash added in Series II amounted to only 4·8, 1·8 and 0·04 per cent. respectively of those already present in Detmer's solution.

The numbers obtained during the first six weeks are shown graphically below, together with a diagrammatic representation of the maximum percentage addition of nitrogen, phosphorus and potash to the quantities present in Detmer's solution.

A photograph of the whole set of 50 dishes, taken during the 6th week, is shown on Plate 21.

A comparison between the relative numbers and weights of plants in all the series is more readily obtained by putting the average number and weight of plants in Series I at 100 for each week, and ascertaining the corresponding figures, which show the percentage of increase, for the other series. These are shown in the Table below.


Series I
II
III
IV
V

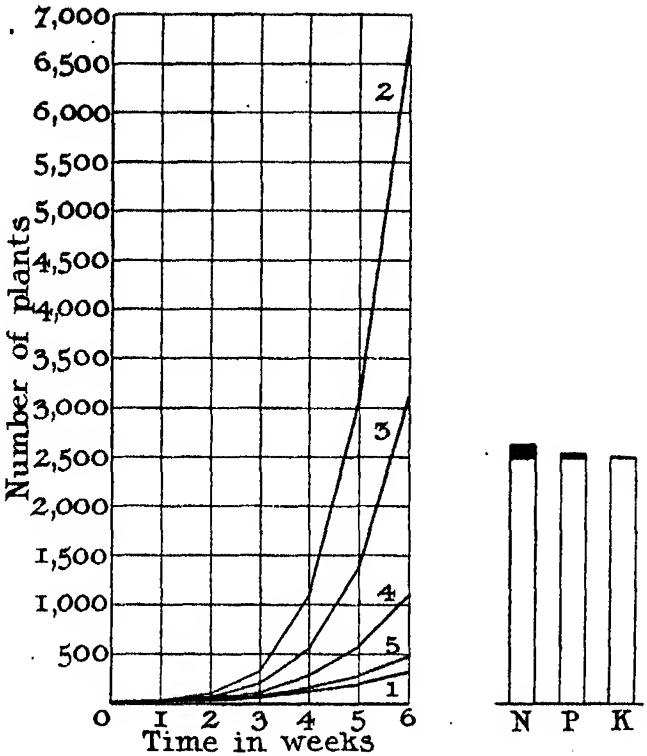


FIG. 1

1. Detmer's solution.
2. " " + water extract.
3. " " + water extract (free from humic acid).
4. " " + alcoholic extract.
5. " " + phosphotungstic fraction.

(The black portions at the top of each column represent the maximum percentage addition (Series II) of N (4·8 per cent.), P (1·8 per cent.), and K (0·04 per cent.), to the quantities already present in Detmer's solution.)

Table IV.

Series.	At beginning of experiment.	1st week.	2nd week.	3rd week.		4th week.		5th week.		6th week.		7th week.	8th week.
		No.	No.	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.	No.
I	100	100	100	100	100	100	100	100	100	100	100	100	100
II	100	128	195	426	536	814	1,519	1,451	4,100	2,059	6,267	3,590	6,587
III	100	117	145	262	288	415	690	660	1,711	957	2,934	1,774	3,684
IV	100	109	114	159	166	207	255	256	492	337	736	580	1,282
V	100	107	107	121	122	126	164	137	223	148	260	177	215

A graphical representation of these figures is shown in the following diagram:

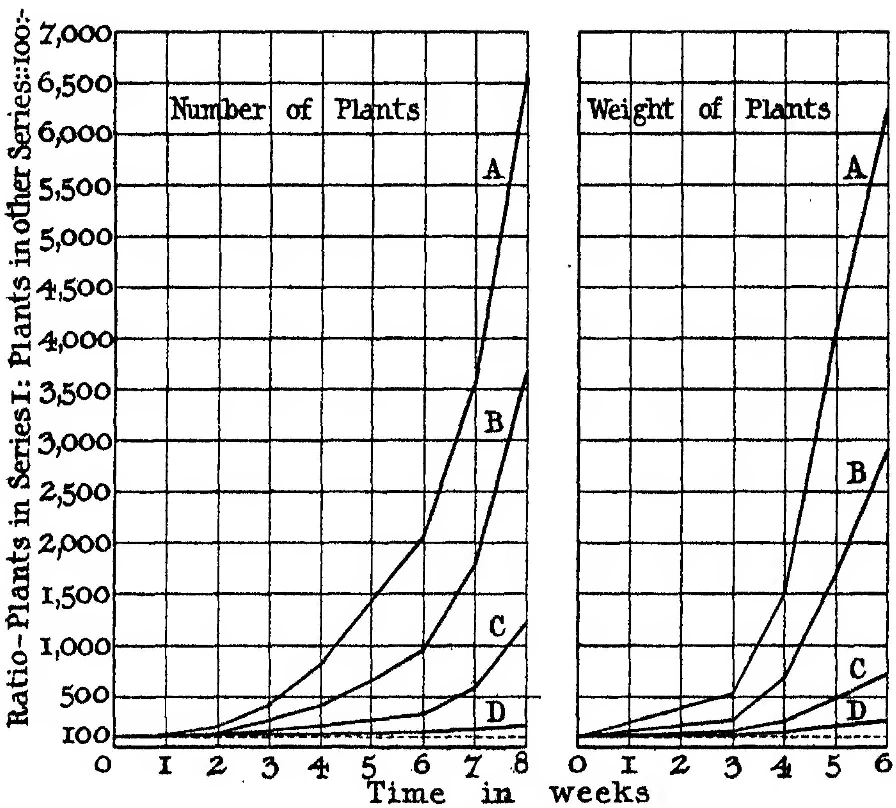


FIG. 2.

A. Series II: Detmer + water extract of bacterised peat.
B. „ III: „ + water extract free from humic acid.
C. „ IV: „ + alcoholic extract.
D. „ V: „ + phosphotungstic fraction.

The marked superiority in size, as indicated by weight, of the plants supplied with auximones is shown in the Table below. The mean weight of five sets, each consisting of 100 plants, at the beginning of the experiment was 12 mg., and the corresponding weights of 100 plants in every series was calculated from the third week onwards, from the average weights and average numbers obtained from all ten dishes in each series.

Table V.

Series.	At beginning of experiment.	3rd week.	4th week.	5th week.	6th week.
	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.
I	12·0	13·0	8·8	5·9	5·4
II	12·0	16·4	16·5	16·7	16·4
III	12·0	14·3	14·7	15·3	16·5
IV	12·0	13·6	10·8	11·3	11·8
V	12·0	13·1	11·5	9·6	9·0

These figures are graphically represented in the diagram below.

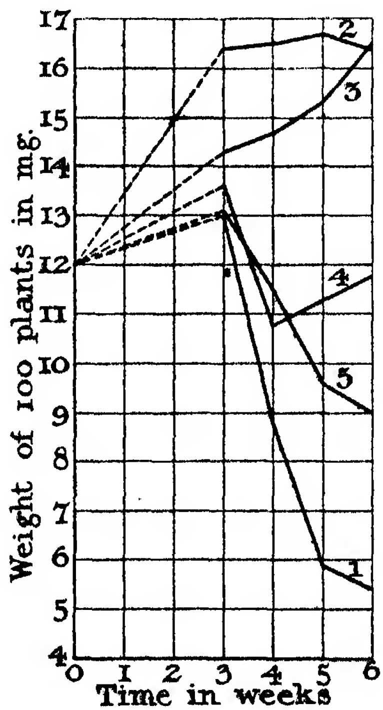


Fig. 3.

1. Series I: Detmer's solution.
2. „ II: „ „ + complete water extract.
3. „ III: „ „ + water extract free from humic acid.
4. „ IV: „ „ + alcoholic extract.
5. „ V: „ „ + phosphotungstic fraction.

A measure of the comparative rapidity with which the plants in each series doubled their number and their weight is obtained by putting the number of times which the plants of Series I doubled themselves in a given time at

unity, and calculating the corresponding figures for each of the other series. The figures thus obtained are given below.

Table VI.

Series.	Comparative rate of doubling in a given time.		Units of time required to double.	
	No.	Wt.	No.	Wt.
I	1·0	1·0	100	100
II	2·05	3·09	48	32
III	1·91	2·71	52	37
IV	1·63	2·01	61	50
V	1·24	1·48	80	67

Very little difference was noticed in the appearance of the plants in the various dishes for the first week, but during the second week the effect of the auximones became apparent, especially in Series II and III, although it was not until the third week that the effect in Series IV and V became well marked. The plants in Detmer's solution gradually assumed a starved appearance which became very marked as the experiment progressed. They gradually lost their green colour, and after the third week their decrease in size was very noticeable. The auximone effect was shown by increased rapidity of multiplication of the plants, larger size, stronger root development and greener colour; that is, essentially, by a more vigorous and healthy growth.

It is clearly evident from the results obtained in the various series that successive fractionation of the extracts obtained from bacterised peat resulted in a diminution of the effective growth-promoting substances present. The best results were shown in Series II, where the whole of the water-soluble substances were supplied, while in Series III, where soluble humate was eliminated from this water extract, growth was not so good. This may have been due partly to the absence of soluble humate as such and partly to the removal of certain organic substances adsorbed in the precipitated calcium humate. Again, in Series IV, the elimination of all except the alcohol-soluble substances resulted in a much less marked, though still very appreciable, effect; and this was still further emphasised in Series V, where the decomposed phosphotungstic-acid fraction was employed. These facts are brought out in the following diagram, in which the five verticals are drawn to represent the five series shown in Table III. The total mean number and weight of the plants in Series I at the end of six weeks are taken to represent unity, and this is marked off on the first ordinate, the corresponding numbers for the other series being calculated, both for weight and number, and marked off on

the successive ordinates. Upon joining up these points, two steadily rising curves are obtained for the numbers and weights, corresponding to an ascending order of complexity in the fractions supplied.

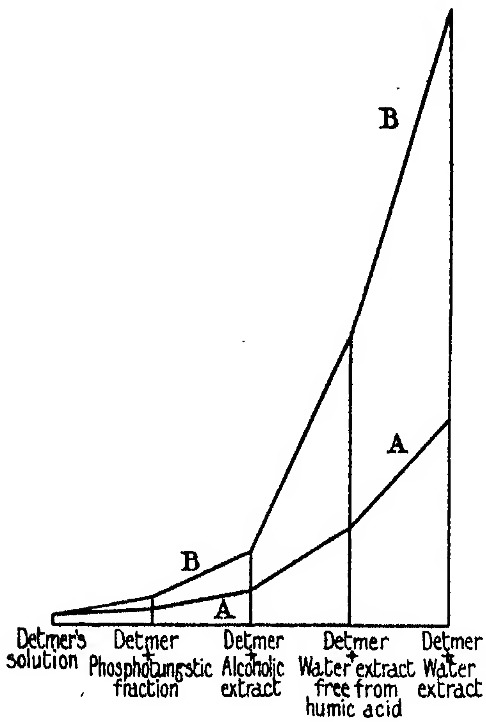


FIG. 4.

It is of interest here to compare the effect of auximones in the 1915 experiments with that shown above. The only comparable figures are those obtained with the phosphotungstic fraction for the first seven weeks of each experiment, since no figures are available for the 8th week. The figures for this period of the experiments shown in Table III give a percentage increase in number over the control plants of 77·4, while the preceding year's figures show an increase of 64·5 per cent. for the corresponding period. Allowing for the variation in conditions of weather and environment it is thus evident that there is a close agreement between the results obtained during the two seasons.

Experiments with Conductivity Water.

In view of the well-known fact that ordinary distilled water such as is commonly used in the laboratory contains traces of toxic substances, it was

thought possible that some of the beneficial effects of the added organic matter in the previous experiments might be due to a neutralisation of the toxicity of the distilled water used.

Owing to the limited supply of glass-distilled or "conductivity" water available, it was impossible to duplicate the whole of the series, but for the purpose of testing how far the diminution in size and vigour of the plants grown in ordinary distilled water, in Series I of the previous experiment, was due to any toxicity of this water, an additional series of ten dishes, precisely similar to Series I, except for the fact that conductivity water was used instead of the ordinary distilled water, was prepared, and this ran concurrently with the rest of the series. This set was numbered from 51 to 60, and was known as Series VI.

The fact that these plants multiplied more rapidly and retained their green colour longer than those in Series I indicated that this water was less toxic than the ordinary distilled water. By the third week, however, when the first halving of the whole set was made, the diminution in size of these plants was very marked. It was therefore decided that, instead of estimating the dry weight of one-half of the plants in each dish in this series at this stage, these plants should be used for testing the effect in conductivity water of certain auximones. As only sufficient conductivity water was available for another ten dishes, and it was desirable that two of the auximone fractions should be tested, two small series, each consisting of five dishes only, were employed. This reduction in the number of the dishes in the new sets appeared to be justified at this stage by the uniformity in the number of plants which prevailed throughout the whole of the dishes in any one series of the above experiment.

The halves of dishes 51 to 55 respectively were transferred to dishes numbered 61 to 65, containing a solution similar to that used in Series III, but made up in conductivity water. This set formed Series VII. Similarly, the halves of dishes numbered 56 to 60 formed another series containing an alcoholic extract of bacterised peat as in Series IV. Unfortunately, the plants in this series very soon became attacked by a fungal disease, and were eventually discarded. At the 7th week of the experiment this series was replaced by another consisting of the halves of the contents of dishes 56 to 60 at this date. This set was numbered from 66 to 70 and formed Series VIII, containing a culture solution similar to that in Series II, made up in conductivity water. The peat extract used here, however, was reduced to half strength, the extract from 1 grm. being used in every 1000 c.c., instead of 500 c.c. as in Series II. This represented a concentration of 184 parts per million of organic matter. These series in conductivity water

Table VII.

Numbers.	Dish 51.	Dish 52.	Dish 53.	Dish 54.	Dish 55.	Dish 56.	Dish 57.	Dish 58.	Dish 59.	Dish 60.	Mean.
At beginning ...	20	20	20	20	20	20	20	20	20	20	20
1st week	22	24	20	21	20	21	20	22	21	22	21 ·3
2nd "	52	51	50	44	46	47	53	53	55	54	50 ·5
3rd "	118	106	118	110	114	102	114	100	126	108	111 ·6
4th "	232	212	236	204	264	208	240	212	252	220	228 ·0
5th "	376	368	472	360	432	352	464	336	464	352	397 ·6
6th "	704	672	784	544	736	608	784	528	816	656	683 ·2
7th "	1,600	1,312	2,016	928	1,728	1,568	1,888	1,088	1,888	1,216	1,523 ·2
8th "	3,008	1,728	2,624	1,344	2,240	2,816	3,392	1,408	3,328	2,048	2,393 ·6
9th "	4,864	2,816	3,712	2,816	3,712	3,200	4,480	3,072	4,992	3,840	3,750 ·4
10th "	6,400	6,656	6,656	5,632	4,608	5,632	6,656	4,864	7,680	5,632	6,041 ·6
11th "	13,824	12,288	12,288	12,800	8,704	11,776	14,336	9,728	14,336	10,752	12,083 ·2
12th "	23,552	20,480	27,648	19,456	14,336	23,552	24,576	20,480	25,600	16,384	21,606 ·4
13th "	49,152	40,960	55,296	47,104	30,720	47,104	47,104	45,056	53,248	34,816	45,056 ·0
Weight—	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.
At beginning ...	2 ·4	2 ·4	2 ·4	2 ·4	2 ·4	2 ·4	2 ·4	2 ·4	2 ·4	2 ·4	2 ·4
4th week	16 ·8	13 ·6	20 ·8	23 ·2	24 ·8	16 ·0	18 ·4	15 ·2	28 ·0	20 ·8	19 ·8
5th "	36 ·8	32 ·0	43 ·2	30 ·4	41 ·6	30 ·4	33 ·6	35 ·2	38 ·4	41 ·6	36 ·3
6th "	60 ·8	57 ·6	70 ·4	54 ·4	64 ·0	60 ·8	70 ·4	60 ·8	76 ·8	48 ·0	62 ·4
7th "	115 ·2	121 ·6	179 ·2	76 ·8	140 ·8	—	—	—	—	—	126 ·7
8th "	204 ·8	208 ·8	211 ·2	83 ·2	166 ·4	224 ·0	200 ·8	121 ·6	211 ·2	134 ·4	176 ·6
9th "	258 ·4	307 ·2	307 ·2	179 ·2	204 ·8	279 ·2	204 ·8	153 ·6	307 ·2	179 ·2	238 ·1
10th "	409 ·6	358 ·4	409 ·6	460 ·8	256 ·0	460 ·8	409 ·6	409 ·6	665 ·6	256 ·0	409 ·6
11th "	1,126 ·4	921 ·6	614 ·4	819 ·2	512 ·0	819 ·2	1,024 ·0	512 ·0	921 ·6	614 ·4	788 ·5
12th "	1,228 ·8	1,843 ·2	1,638 ·4	1,433 ·6	614 ·4	1,228 ·8	1,024 ·0	1,843 ·2	1,843 ·2	614 ·4	1,331 ·2
13th "	3,686 ·4	2,867 ·2	2,048 ·0	2,048 ·0	2,867 ·2	3,686 ·4	1,228 ·8	2,457 ·6	2,457 ·6	2,048 ·0	2,539 ·5

ran until the 13th week from the commencement of Series VI, and the figures which would then have been obtained from each complete set of 20 plants in Series VI are shown in the Table on the preceding page.

The corresponding numbers for the five dishes in Series VII can be given for only 11 weeks instead of 13, since this was not started until the 3rd week; and it should be noted that the numbers in the dishes of this series at the beginning of the experiment are not uniformly 20 as in the previous series, but show a variation, for they represent the halves of the contents of dishes 51 to 55 respectively of Series VI in its 3rd week.

Table VIII.

	Dish 61.	Dish 62.	Dish 63.	Dish 64.	Dish 65.	Mean.
Number of plants—						
3rd week (at beginning)	59	53	59	55	57	56 ·6
4th week	128	122	138	128	130	129 ·2
5th "	288	288	292	284	308	292 ·0
6th "	592	592	608	616	664	614 ·4
7th "	2,144	2,192	1,872	2,192	2,112	2,102 ·4
8th "	7,872	8,640	5,920	9,024	8,064	7,904 ·0
9th "	26,240	20,864	22,272	24,960	24,320	23,731 ·2
10th "	51,456	56,320	50,176	52,736	48,640	51,865 ·6
11th "	110,080	122,880	114,176	110,080	107,008	112,844 ·8
12th "	201,728	222,208	228,352	215,040	211,968	215,859 ·2
13th "	460,800	487,424	538,624	460,800	454,656	480,460 ·8
Weight of plants—	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.
4th week	10 ·8	11 ·6	14 ·0	12 ·4	12 ·4	12 ·2
5th "	38 ·4	36 ·0	36 ·8	33 ·6	34 ·4	35 ·8
6th "	81 ·6	86 ·4	96 ·0	84 ·8	84 ·8	86 ·7
7th "	297 ·6	313 ·6	265 ·6	288 ·0	313 ·6	295 ·7
8th "	1,004 ·8	1,146 ·6	851 ·2	1,017 ·6	1,011 ·2	1,006 ·1
9th "	2,982 ·4	2,867 ·2	2,944 ·0	2,713 ·6	2,841 ·6	2,869 ·8
10th "	5,734 ·4	7,270 ·4	6,451 ·2	7,065 ·6	6,451 ·2	6,594 ·6
11th "	14,848 ·0	16,998 ·4	15,667 ·2	17,100 ·8	14,745 ·6	15,872 ·0
12th "	28,876 ·8	36,249 ·6	36,454 ·4	33,996 ·8	33,587 ·2	33,832 ·9
13th "	68,403 ·2	81,100 ·8	87,654 ·4	70,041 ·6	78,233 ·6	77,086 ·7

Similarly in the case of Series VIII the figures, shown in the Table below, can be given for seven weeks only, since this series was not commenced until the 7th week of the whole experiment (Table IX).

A correct comparison of the rates of growth in these three series cannot be obtained by comparing the figures in the columns for corresponding weeks, since the three series were all started at different dates. The comparison can only be made by putting the number of plants in Series VI at 100 for each week, and calculating the corresponding numbers for Series VII and VIII. It must be noted, however, that Series VII was started at the 3rd week of Series VI by taking one-half of the contents of the dishes of

Table IX.

	Dish 66.	Dish 67.	Dish 68.	Dish 69.	Dish 70.	Mean.
Number of plants—						
7th week (at beginning)	49	59	34	59	38	47·8
8th week	138	204	106	180	90	143·6
9th "	524	680	444	648	480	555·2
10th "	1,056	1,344	976	1,360	840	1,115·2
11th "	2,944	3,232	2,208	3,792	2,128	2,860·8
12th "	6,272	7,360	4,320	5,920	4,160	5,606·4
13th "	16,576	19,008	12,800	22,656	10,944	16,396·8
Weight of plants—	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.
8th week	16·0	19·6	9·6	16·0	11·2	14·5
9th "	56·0	66·4	52·0	67·2	45·6	57·4
10th "	123·2	142·4	116·8	148·8	83·2	112·9
11th "	355·2	412·8	300·8	489·6	272·0	366·1
12th "	1,004·8	1,126·4	736·0	953·6	652·8	894·7
13th "	2,624·0	2,764·8	2,086·4	3,648·0	1,817·6	2,588·2

Series VI to start the new series. The numbers given in the Table for Series VI, however, represent not the halves which were left and which would correspond with the new series, but the complete sets which would have been obtained had the contents of the dishes not been halved. It is evident, therefore, that the numbers for Series VII from the 3rd week onwards correspond to one-half of the numbers given for Series VI for the same period, and that, in order to obtain a correct comparison, one-half of the mean numbers for Series VI must be put at 100, and the corresponding numbers for Series VII then calculated. Similarly, Series VIII was started at the 7th week of Series VI, at the fifth time of halving these dishes. One thirty-second part of the complete sets for Series VI was therefore used to start Series VIII, and the numbers for the latter series from the 7th week onwards correspond to 1/32 of the numbers given for Series VI. In order therefore to correctly compare the rate of growth in these two series, the mean numbers for Series VI must be divided by 32, and the resulting figures put at 100, to obtain the corresponding numbers for Series VIII. A comparison thus obtained for both numbers and weights is given in Table X on p. 498.

The average weekly weight of 100 plants in each of the three series is shown in Table XI on p. 498.

The drop in the average weight of the plants in Series VII from the 7th to the 9th week is probably explained by the excessive multiplication of the plants during a fortnight of extremely hot weather An abnormally large number of small new plants were thus included in the weekly countings, and this resulted in a smaller average weight.

Table X.

Series.		3rd week.	4th week.	5th week.	6th week.	7th week.	8th week.	9th week.	10th week.	11th week.	12th week.	13th week.
VI. Detmer	No.	100	100	100	100	100	100	100	100	100	100	100
	Wt.	100	100	100	100	100	100	100	100	100	100	100
VII. Detmer + water extract free from humic acid	No.	100	113	147	179	276	660	1265	1716	1867	1998	2133
	Wt.	100	123	198	278	467	1189	2411	3220	4026	5083	6071
VIII. Detmer + water extract	No.	—	—	—	—	100	192	466	591	759	852	1164
	Wt.	—	—	—	—	100	263	771	882	1486	2150	3261

Table XI.

	Weight of 100 plants.										
	At beginning.	4th week.	5th week.	6th week.	7th week.	8th week.	9th week.	10th week.	11th week.	12th week.	13th week.
	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.	mgrm.
Series VI: Detmer's solution	12·0	8·7	9·1	9·1	8·3	7·4	6·3	6·8	6·5	6·1	5·6
Series VII: Detmer + water extract free from humic acid	—	9·5	12·3	14·1	14·1	12·7	12·1	12·7	14·0	15·7	16·0
Series VIII: Detmer + water extract	—	—	—	—	—	10·1	10·3	11·0	12·8	15·9	15·8

It is evident from these figures that, whilst the toxic substances in the metal-distilled water employed in the previous experiments has had a certain injurious effect upon the growth of the plants, yet the use of pure non-toxic water with mineral salts will not suffice for normal and healthy growth for any length of time. Although the plants in conductivity water multiplied more rapidly, retained their healthy appearance longer, and decreased in weight less rapidly than those in ordinary distilled water, yet the final result in each case was the same.

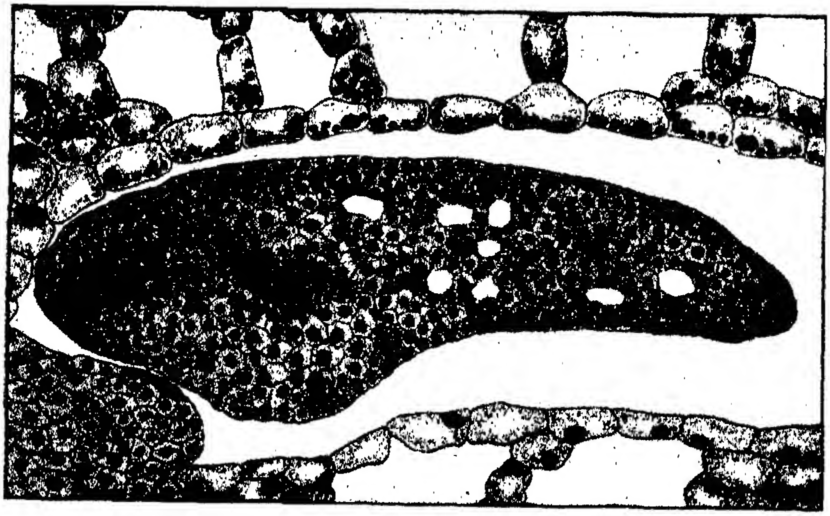
These figures are graphically represented in the diagram, fig. 5.

In view of the striking differences in general appearance between the control and auximone plants in both ordinary distilled water and conductivity water, an investigation was made of the internal structure of representative plants from each set at the conclusion of the experiment. The plants were fixed, microtomed and stained in the usual manner. Microscopic examination then showed that in all the plants examined receiving auximones, the tissues were more dense, and the proportion of air spaces to

Section through young frond. Series VI. × 235.

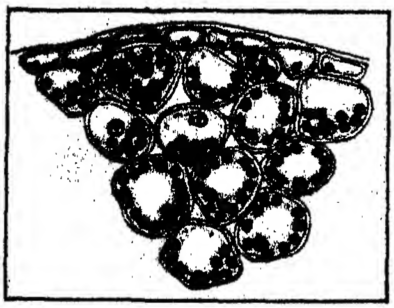
− Auximones.

Section through young frond. Series VIII. × 235.



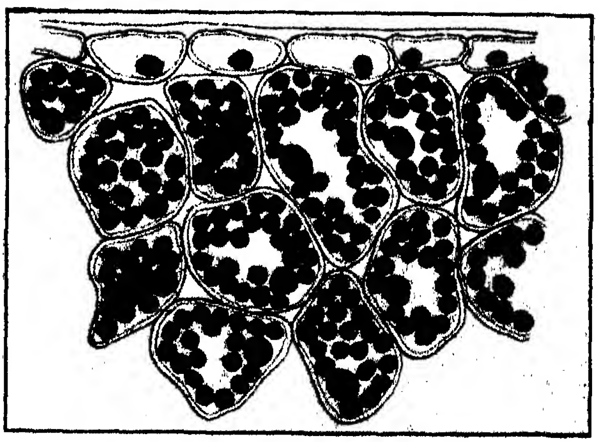
+ Auximones.

Typical cells from old frond. Series VI. × 610.



− Auximones.

Typical cells from old frond. Series VIII. × 610.



+ Auximones.

cellular tissue was much less than in the control plants. The difference was also very evident in the individual cells. In the auximone plants they were

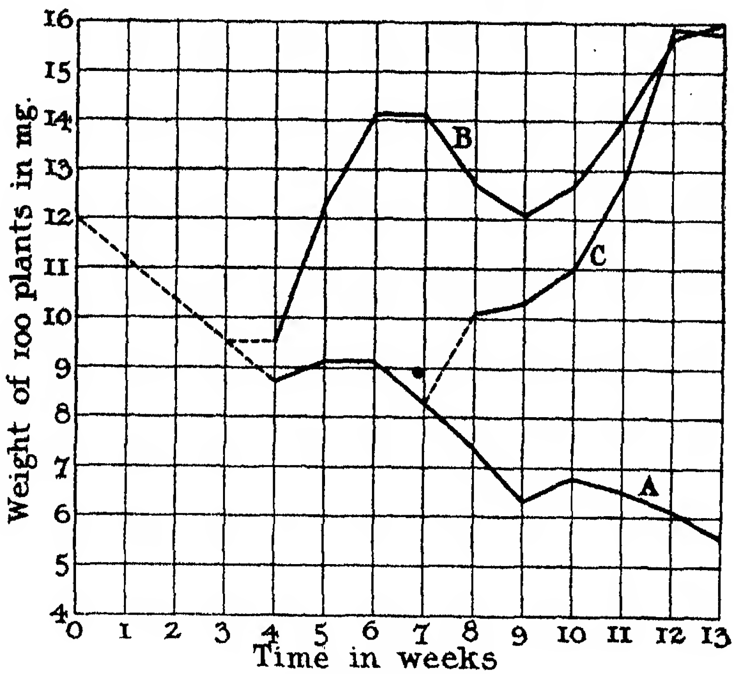


FIG. 5.

A. Series VI: Detmer's solution in conductivity water.
B. „ VII: „ „ + water extract free from humic acid.
C. „ VIII: „ „ + complete water extract.

larger and more densely filled with protoplasm, containing larger nuclei and more numerous and larger chloroplastids than in the control plants. This difference was especially noticeable at the apex of young, newly-formed plants.

These various points are evident from the diagrams on Plate 22, drawn with the camera lucida, of representative plants grown in Series VI and VIII in conductivity water, the two of each set being of the same magnification.

Interchange of Culture Solutions.

When it became evident from the previous experiments that a period of three weeks generally elapsed before the effect of added auximones became very marked in plants which had been taken from their normal habitat, a further experiment was commenced at King's College to ascertain how soon plants grown in water culture with and without auximones would respond to a reversal of these conditions.

Two series of five dishes each were prepared containing solutions similar to those in Series I and II above. The dishes were numbered from 1 to 10, Nos. 1 to 5 forming Series I with Detmer's solution, and Nos. 6 to 10 Series II with Detmer's solution plus complete water extract. Twenty uniform plants of *Lemna minor* were placed in each dish, and the sets treated precisely as in the preceding experiments for five weeks. Twenty average plants were then counted out from each dish and the culture solutions in each series were transposed; that is, the five sets each of 20 plants previously growing in Detmer's solution were now in Detmer's solution plus auximones (Series I), while those hitherto supplied with auximones were now growing in Detmer's solution alone (Series II). These plants were allowed to grow on for another nine weeks, receiving the same treatment as to changing and counting as before. At this stage 20 plants from each series were again transposed, that is, were transferred to their *original* solutions, and these were counted for a further six weeks. The numbers in the five dishes of each series approximated very closely to the mean for the series throughout, so only the average numbers are given in the Table below:—

Table XII.

Period I.			Period II.			Period III.		
	Series I. − Auximone.	Series II. + Auximone.		Series I. + Auximone.	Series II. − Auximone.		Series I. − Auximone.	Series II. + Auximone.
At beginning	20	20	5th week (at beginning)	20	20	14th week (at beginning)	20	20
1st week	30	46	6th week ...	49	58	15th week ...	97	89
2nd "	64	104	7th " ...	102	113	16th " ...	402	458
3rd "	122	235	8th " ...	201	201	17th " ...	722	957
4th "	192	680	9th " ...	688	490	18th " ...	1,704	2,689
5th "	426	1,528	10th " ...	2,848	1,212	19th " ...	3,288	5,606
			11th " ...	7,552	2,956	20th " ...	7,648	14,720
			12th " ...	23,910	6,707			
			13th " ...	108,953	23,142			
			14th " ...	372,531	73,818			

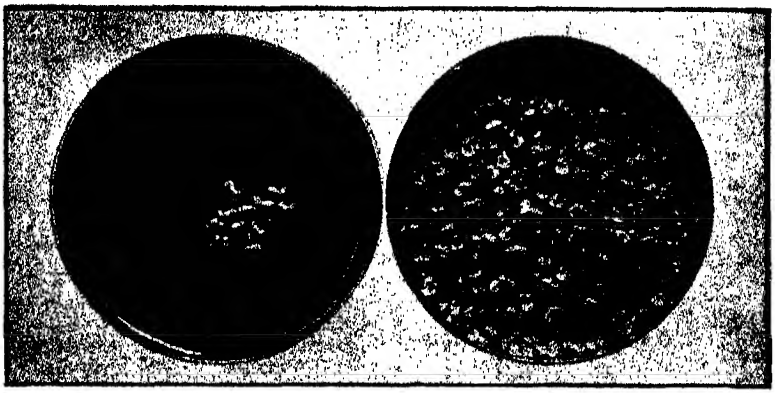
It is very evident from the above figures that whenever auximones are present in the culture solutions the plants multiply much more rapidly than in mineral nutrients alone. This is brought out by a comparison of the final figures for each period.

Period I.	Detmer − auximones	426	Detmer + auximones	1,528
Period II.	Detmer + auximones	372,531	Detmer − auximones	73,818
Period III.	Detmer − auximones	7,648	Detmer + auximones	14,720

This comparison is also well shown by the following photographs of one typical dish from each series taken during Periods I and II respectively:—

PERIOD I.

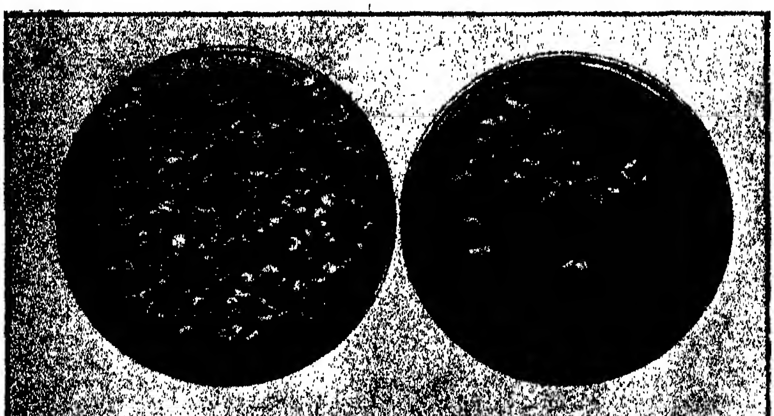
Series I. Series II.



– Auximones. + Auximones.

PERIOD II.

Series I. Series II.



+ Auximones. – Auximones.

FIG. 6.

A comparison of the rate of growth in the different culture solutions is best obtained as before by putting the average number of plants in Series I at 100 for each week, and estimating the corresponding numbers for Series II. The figures thus obtained are:—

Table XIII.

Series I.	Series II (1st period).		Series II (2nd period).		Series II (3rd period).	
100 throughout ...	At beginning	100	5th week .... (at beginning)	100	14th week .... (at beginning)	100
	1st week......	152	6th week	118	15th week	92
	2nd "	162	7th "	111	16th "	114
	3rd "	193	8th "	100	17th "	132
	4th "	326	9th "	71	18th "	158
	5th "	359	10th "	52	19th "	170
			11th "	39	20th "	192
			12th "	28		
			13th "	21		
			14th "	20		

These figures are represented graphically in the diagram below:—

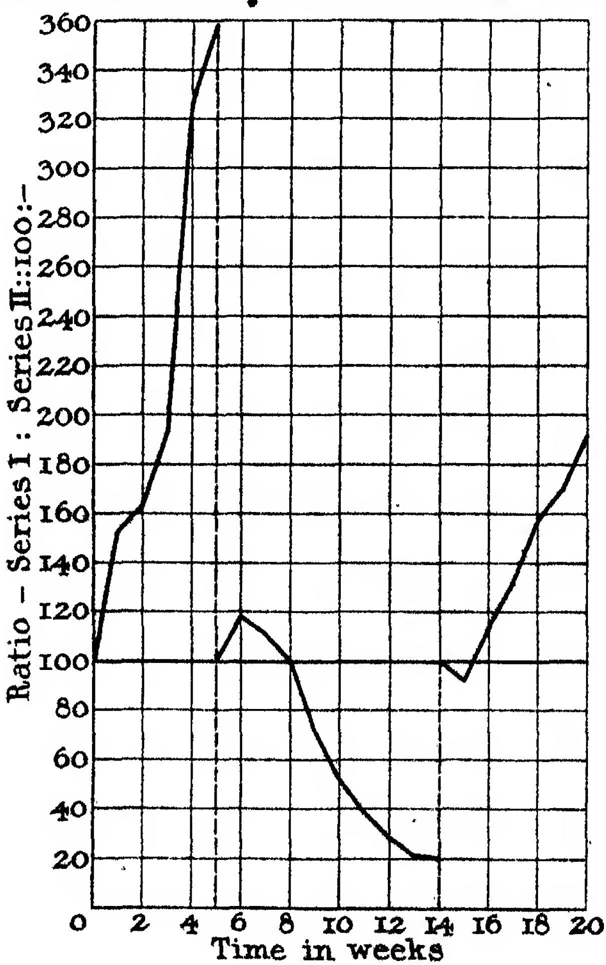


Fig. 7.

From this diagram it is seen that plants which have been growing for some time in either Detmer's solution or Detmer's solution plus auximones do not respond immediately to the addition or removal of these substances to or from the culture solution. There is about a week—a "reaction time"—during which the previous tendency to decrease or increase is manifest before the new factor becomes effective.

An important point, which was noticed throughout the experiments, was the fact that the greatest variation in the numbers and weights of the plants in the various dishes each week appears in the control sets. Evidently, where the plants are lacking auximones, there is a lack of uniformity between the rate of multiplication and the increase in weight of the plants. In some weeks growth-energy appears to be expended in multiplication, with little corresponding increase in weight; in other weeks increase in weight appears to be attained at the expense of numbers. For example, in Table VI, Series VI, Dish 58 in conductivity water shows a doubling of number from the 10th to the 11th week, but the weight has only increased by about 25 per cent. during the same period. From the 11th to the 12th week the same dish shows a further increase of rather more than 100 per cent. in the number, but this time it is accompanied by an increase of more than 250 per cent. on the weight. Similarly, in Table II, Series II, Dish 4 in ordinary distilled water shows an increase in number of practically 50 per cent. from the 4th to the 5th week, but the weight has scarcely increased at all, while from the 5th to the 6th week, an increase in number of about 60 per cent. is accompanied by an increase in weight of about 50 per cent.

In the series containing auximones there is a much greater conformity between the rate of multiplication and increase in weight.

Conclusions.

The foregoing experiments appear to justify the conclusions (1) that bacterised peat contains certain organic substances which, when supplied even in small quantities to *Lemna* plants growing in complete mineral culture solutions, have a remarkable effect upon their growth; (2) that in these plants normal growth and multiplication cannot be sustained for any length of time in the absence of these organic growth-promoting substances or auximones; (3) that these substances are essential for the effective utilisation and assimilation of the mineral nutrients supplied to these plants.

It is highly probable that these auximones are organic decomposition products, for bacterised peat is simply organic matter, already partially decomposed by anaërobic action, which has been further decomposed by the

agency of aërobic bacteria under suitable conditions. Hence the active growth-promoting substances obtained in solution from this must be products of decomposition of organic matter. Further experiments, not yet published, have shown that these substances are also obtainable, in relatively small amounts, from well-rotted stable manure and thoroughly decomposed leaf-mould. It is to be expected, therefore, that small quantities would be formed among the products of organic decay in the water of a pond, and it is interesting to note, in this connection, that in nature *Lemna* plants flourish best in stagnant water, rich in organic decomposition products.

Until more is known as to the nature of these products, it is impossible to state definitely how they function. Some of them may be absorbed and utilised directly as plant nutrients. Schreiner and Skinner* have shown that such nitrogenous decomposition products as creatinine, histidine and arginine can replace nitrates in a culture solution, and that, even when nitrates are present, these substances are absorbed by the plants. They say that "these compounds are absorbed as such, and utilised directly for building up the proteins and other complex nitrogenous constituents of vegetable material." They also suggest that the energy usually employed in a plant in effecting the chemical transformation of inorganic nitrogen into an organic form can be expended otherwise when these substances are supplied to the plant, and thus "plant efficiency is increased and growth augmented when the plant obtains compounds which will serve directly as tissue builders."

On the other hand, some may have a similar effect to that of the accessory food bodies or growth vitamines concerned in animal growth. The phosphotungstic fraction of bacterised peat was obtained by precisely the same method as that used for extracting animal growth vitamines, and it is difficult to understand how the addition of such a small amount of organic matter as 13 parts per million from this material to a culture solution already containing 5500 parts per million of mineral nutrient salts could produce the results obtained if it represented only a further addition of plant nutrient. The possibility that such accessory food bodies are present in bacterised peat is emphasised by the fact that the water extract free from humic acid, the alcoholic extract, and the phosphotungstic fraction, have all been found to give a positive reaction with the Folin-Macullum phosphotungstic acid reagent. This positive reaction, which is not given by the corresponding fractions of raw peat, is considered by Williams and

* Schreiner and Skinner, U.S. Dept. Agric., Bureau of Soils, Bull. 87, 1912.

Seidell* to indicate, with certain reservations, the presence of animal growth vitamines.

It is possible that both organic plant nutrients and accessory food substances were present in the complex water extract of bacterised peat supplied in the above experiments, and that these had a co-operative effect, for, even if one assumes that the phosphotungstic fraction contained only accessory food substances, it is seen that the more complex water extract produces the best growth results.

In view of the generally accepted botanical theory that green plants can build up complex protein compounds from mineral salts, and mineral salts only, the above results are difficult to explain. If this theory be correct, then *Lemna minor* must either have lost this power, or it is an exception to the general rule, since it is evident that, for healthy growth, this plant must be supplied with small quantities of certain organic substances in addition to mineral nutrients. The beneficial effect of organic manures in cultural operations is well known. Recently Livingston† has shown that manure extract and the expressed juice of red-clover leaves have a remarkable effect in increasing the growth of wheat seedlings in culture solutions consisting of soil extracts and mineral nutrients. He demonstrates that it is the organic matter of the extracts which is beneficial, but he attributes the benefit to "some correcting influence which it brought to bear upon the toxic bodies of the soil extract and those which appear to be produced by the seedlings" rather than to a direct action upon the nutrition of the plants.

The remarkable effect of the addition of organic substances in the experiments on *Lemna minor* recorded above, as manifested not only by the enormous increase in number and weight of the plants, but also by the increased vigour of the individual cells, as shown by the denser protoplasm, larger nuclei and more numerous chloroplastids, indicates that the organic substances play some definite and essential part in the general metabolic activities of the plant.

To what extent certain organic substances may be necessary for the growth of green plants in general, further experiments alone can decide. The well-known fact that the seedlings of land plants can be grown to maturity in culture solutions of mineral salts is not a fatal objection to the suggestion that all green plants may require traces of certain organic substances for their optimum development, since it has been shown that nitrogenous organic growth-promoting substances are produced during the

* Williams and Seidell, 'Jour. Biol. Chem.,' vol. 26, pp. 431–456 (1916).

† Livingston, B. E., U.S. Dept. Agric., Bureau of Soils, Bull. 36, 1907.

germination of seeds. Brown* has shown that during the germination of barley seeds, certain soluble nitrogenous substances, which are essential for the early stages of development of the young plant, are formed in the endosperm and absorbed by the embryo. The writer also has found that a water extract of germinated seeds has a similar effect on *Lemna* plants growing in culture solution to that of bacterised peat, while no growth-promoting substances could be obtained from dry seeds. It is probable, therefore, that the seedlings used in the ordinary water-culture experiments may already contain the necessary minimal quantities of organic substances requisite for ordinary growth, but that a further supply of these substances is essential for optimum growth and development. It is therefore not unreasonable to assume that organic growth-promoting substances are as essential for plant as for animal nutrition, since the difference in metabolism between plants and animals is one of degree only; and that just as the plant is indebted to the bacterial activities of the soil for the nitrogen which it passes on to the animal in elaborated form, so the growth vitamines which the animal obtains from the plant are not entirely manufactured by the plant as such, but are, at least partially, the products of bacterial activity in the soil in which the plants are growing.

Summary.

1. Raw peat, when further decomposed by means of aërobic soil organisms —"bacterised peat"—is found to contain certain growth-promoting substances (auximones).

2. *Lemna minor* plants cannot maintain growth for any length of time in culture solutions containing only mineral nutrients.

3. The presence of soluble organic matter is essential for complete growth.

4. The addition to the mineral culture solution of 368 parts per million of organic matter from the water extract of bacterised peat resulted, after six weeks, in a multiplication of the number to 20 times, and an increase in weight to 62 times, that of the control plants. The water extract free from humic acid, representing an addition of 97 parts of organic matter per million, gave 9½ times the number and 29 times the weight; 32 parts per million from the alcoholic extract gave 3⅓ times the number and 7⅓ times the weight; 13 parts per million from the phosphotungstic fraction gave 1½ times the number and 2½ times the weight.

5. The effect of the reduction in amount of auximones with successive fractionation of the bacterised peat was also manifest from the general appearance of the plants. Those in mineral nutrients only decreased in size

* Brown, H. T., 'Trans. Guinness Research Lab.,' vol. 1, p. 288 *et seq.* (1906).

week by week, and became very unhealthy in appearance, whilst there was a progressive improvement in the appearance of the plants supplied with increasing amounts of auximones. Those receiving the larger amounts retained their normal healthy appearance throughout the experiment and increased in size.

6. The beneficial effect of the auximones was not due to a neutralisation of the toxic substances present in the ordinary distilled water, since comparable results were obtained with conductivity water.

7. An interchange of culture solutions, with and without auximones, showed that the plants are very sensitive to the presence or absence of these substances.

8. It is suggested that some of these growth-promoting substances may act directly as organic nutrients, and others may be of the nature of accessory food substances.

I am indebted to the authorities of the Botanical Department, Imperial College of Science and Technology, South Kensington, for facilities afforded for carrying out a considerable portion of the work in their greenhouse laboratory. I wish also to express my grateful thanks to my assistant, Miss Mockeridge, B.Sc. Without her valuable help it would have been impossible to obtain a record of the numbers and weights of the plants each week.

An Application of the Theory of Probabilities to the Study of a priori *Pathometry.*—Part II.

By Lieut.-Colonel Sir RONALD ROSS, K.C.B., F.R.S., R.A.M.C.T.F., and HILDA P. HUDSON, M.A., Sc.D.

An Application of the Theory of Probabilities to the Study of a priori *Pathometry.*—Part III.

By Lieut.-Colonel Sir RONALD ROSS, K.C.B., F.R.S., R.A.M.C.T.F., and HILDA P. HUDSON, M.A., Sc.D.

[The above papers are published in 'Proceedings,' Series A, vol. 93, No. 650.]

Some Effects of Organic Growth-Promoting Substances (Auximones) on the Soil Organisms concerned in the Nitrogen Cycle.

By Florence A. Mockeridge, B.Sc., King's College, London.

(Communicated by F. W. Oliver, F.R.S. Received January 2, 1917.)

It is a well-established fact that the presence of a certain amount of humus in soil is essential to complete fertility, and it is equally well known that this organic matter, whether supplied in the form of stable or green manure, is far more effective when decomposed, or "rotted," than when fresh. This has been attributed to the soluble humus formed during the rotting, but wherein lies the peculiar merit of this soluble humus has long been a debatable point, some considering that it serves primarily as a plant nutrient, while others claim that its most important effect is upon the bacterial flora of the soil.

It is well known that raw peat, although rich in humus, is practically useless as a manure, on account of its acid and insoluble nature. This insoluble humus can be neutralised and rendered largely soluble by extraction with alkalies, and almost all experiments on the effect of soluble humus on bacteria have hitherto been carried out with such extracts. Too much reliance must not be placed on the results obtained with these chemically prepared substances, for they are not strictly comparable with the soluble humus produced in the soil by natural processes. However, Bottomley* has shown that it is possible, by inoculating peat with certain aërobic soil bacteria, and keeping it under suitable conditions, to convert it into a partially soluble humus in a comparatively short time. A similar bacterial action is taking place more slowly in every rotting manure heap and in all soils, for the longer stable and farm manures are kept, the more water-soluble brown humus can be extracted from them. Bottomley's bacterial treatment of the peat simply reproduces and hastens these natural processes, and the product of the treatment, which is known as "bacterised peat," is practically the counterpart, in a more concentrated form, of rotting stable manure or of green manures which are undergoing decomposition in the soil. The soluble humus which can be extracted from it may be justly considered to approximate more closely to the natural product than any extracts obtained by chemical processes, and the

* Bottomley, W. B., 'Journ. Roy. Soc. Arts,' vol. 62, No. 3199 (1914).

effects of this soluble humus on soil bacteria accordingly reproduce more exactly the influence of the organic matter of soils upon the bacterial flora.

Recently Bottomley* has shown that a water extract of bacterised peat, and certain fractions obtained therefrom, produce an increase in the growth of plants which cannot be attributed to any purely nutritive effect caused by the recognised manurial constituents present, and he suggested that during the decomposition of the peat, certain accessory food substances are formed, which fulfil a similar function to that of the vitamines known to be so important in animal nutrition. His recent work on the influence of these growth-promoting substances obtained from bacterised peat, which he has called "auximones," has lent confirmation to this suggestion, and it was with the object of investigating the effect of these auximones upon the four chief groups of soil organisms concerned in the nitrogen cycle, that is, upon the nitrogen-fixing, the nitrifying, the ammonifying, and the denitrifying bacteria, that the present research was undertaken.

Nitrogen Fixation.

It was first pointed out three years ago by Bottomley† that the addition of bacterised peat to soil results in a marked increase in the rate of nitrogen fixation, and this result was attributed purely to the activity of the nitrogen-fixing organisms introduced into the soil with the material. He‡ has since found that, apart from the organisms which the material contains, the bacterised peat itself has certain inherent properties which have the effect of increasing the rate of nitrogen fixation by soil organisms to a marked degree, and that these properties are not possessed by either raw or chemically treated peat. The experiments hitherto recorded, however, have been more or less isolated, and it appeared advisable to undertake a more extensive investigation of the effect of bacterised peat, and the various fractions obtained from it, upon nitrogen fixation in soil and in both crude and pure liquid culture.

A preliminary experiment was carried out to determine whether bacterised peat which had been sterilised at 135° C., to kill off the nitrogen-fixing organisms which it contained, would stimulate nitrogen fixation in soils. Six portions, each consisting of 24 oz. of a uniform sample of a loamy soil, were weighed out. Two were mixed with one part in ten by volume of sterilised bacterised peat, and two with a similar

* Bottomley, W. B., 'Roy. Soc. Proc.,' B, vol. 88, pp. 237–247 (1914).

† Bottomley, W. B., 'Report Brit. Assoc.,' 1913.

‡ Bottomley, W. B., 'Roy. Soc. Proc.,' B, vol. 88, pp. 237–247 (1914), and vol. 89, pp. 102–108 (1915).

quantity of the normal material containing living organisms, the remaining two portions serving as controls. A similar experiment was also arranged with a clay soil. When three samples had been taken from each for analysis of their nitrogen content, all the soils were placed in large glass bottles loosely corked, so that they could be well shaken daily to ensure aëration, and were placed on top of an incubator kept at 26° C., the average temperature of the soils being about 20–22° C. The moisture content was kept as uniform as possible, and after two weeks' incubation the soils were again sampled, the total nitrogen being determined by the Kjeldahl process. The results obtained were :—

Table I.

	Original nitrogen content (mgrm. N per 100 grm. soil).				Nitrogen content after two weeks (mgrm. N per 100 grm. soil).				Gain in two weeks (mgrm. N per 100 grm. soil).	
	Soil A, Loam.		Soil B, Clay.		Soil A, Loam.		Soil B, Clay.		Soil A, Loam.	Soil B, Clay.
1. Soil alone	220 224 227	224	295 306 299	300	236 230 238	235	313 320 322	318	11	18
2. " "	229 224 225	226	299 302 296	299	237 241 240	239	316 323 319	319	13	20
3. Soil + sterilised bacterised peat	362 372 371	368	465 462 454	460	410 403 400	404	513 509 502	508	36	48
4. " "	375 368 368	370	464 468 462	465	398 405 400	401	504 514 510	509	31	44
5. Soil + normal bacterised peat	368 360 365	364	458 464 465	462	424 430 428	427	527 522 524	524	63	62
6. " "	364 371 372	369	466 458 457	460	432 428 434	431	526 518 517	520	62	60

The three analyses of each soil gave results which approximated very closely to the mean, and therefore, in the following Tables of soil analyses, only the mean of the three determinations will be given in each case, in order to avoid unnecessary figures. The maximum deviation from the mean for nitrogen fixation was ± 6.

It is thus apparent that the addition of bacterised peat has increased the activities of the nitrogen-fixing organisms already in the soil, quite apart from any bacteria introduced. This result may have been due to (1) the physical effect of the organic matter in improving the aëration of the soil, and thus facilitating the activities of the aërobic bacteria; (2) the effect of

the soluble humate alone; (3) the effect of the organic products, other than soluble humate, formed during the bacterisation of the peat. If the first be the case, then a similar result might be expected upon the addition of raw peat to the soil, if the soil be limed to correct the acidity of the peat; and, if the second be true, then chemically treated peat should produce a like result. Accordingly, another six portions of soil from each of the same localities were weighed out, two of each being mixed with one part in ten by volume of raw peat and 1 per cent. of their weight of powdered calcium carbonate. Two other portions of each were mixed with one part in ten by volume of peat which had been treated with 2 per cent. of its weight of sodium carbonate, and which was absolutely neutral, while the remaining two portions served as controls. Since the soils already contained sufficient lime, the chalk was added only to those containing raw peat. The soils were sampled, as before, for their nitrogen content, and then placed on top of the incubator at about 20° C. for a fortnight, at the end of which period they were again analysed, with the following results:—

Table II.

	Original nitrogen content (mgrm. N per 100 grm. soil—mean of three determinations).		Nitrogen content after two weeks (mgrm. per 100 grm.—mean of three determinations).		Gain in two weeks (mgrm. N per 100 grm. soil).	
	Soil A, Loam.	Soil B, Clay.	Soil A, Loam.	Soil B, Clay.	Soil A, Loam.	Soil B, Clay.
1. Soil alone	220	300	230	318	10	18
2. " "	221	299	232	319	11	20
3. Soil + raw peat ...	285	369	273	381	−12	12
4. " " ...	290	371	270	387	−20	16
5. Soil + carbonated peat	292	365	304	382	12	17
6. " "	286	368	300	388	14	20

In these soils it is evident that neither the aëration nor the chemically formed soluble humus are capable of producing an effect comparable with that of the bacterised peat. The addition of raw peat appears to result in one case in a loss of nitrogen, while the chemically treated peat does not appreciably affect the nitrogen-fixing organisms.

These and other results obtained from time to time all tend to show that bacterised peat has the property of increasing nitrogen fixation in soils, independently of the organisms which it contains, and quite apart from any physical effect or any purely stimulating property of the soluble humates.

as such. In order to investigate further this property a fractionation of the bacterised peat was made according to the methods already described by Bottomley,* and the decomposed phosphotungstic-acid fraction obtained. Four portions, each consisting of 40 oz. of soil from Chelsea Physic Garden, were weighed out, and to each of two of them was added, in solution in distilled water, the phosphotungstic fraction of that weight of bacterised peat which, if mixed with the soil, would give a proportion of one part of peat in ten of soil by bulk; the other two served as controls. The four soils were sampled at once for their nitrogen content, and were then placed in loosely corked glass bottles on top of the incubator as before, for about seven weeks, moisture being added when necessary, and the bottles being shaken daily. Samples analysed twice during that period gave the following results :—

Table III.

	Original nitrogen content (mgrm. per 100 grm.—mean of three determinations).	Nitrogen content after 28 days (mean of three determinations).	Nitrogen content after 52 days (mean of three determinations).	Gain in 28 days.	Gain in 52 days.
1. Soil alone	312	321	333	9	21
2. ,, ,,	316	322	335	6	19
3. Soil + phosphotungstic fraction	314	333	373	19	59
4. ,, ,,	317	337	380	20	58

The figures here given show that the addition of even the phosphotungstic fraction to the soil results in an increase in nitrogen fixation, although it is not to be expected that this fraction would be as effective as the bacterised peat itself. This substance certainly contains neither organisms nor soluble humus as such, nor can it have any effect on the physical condition of the soil. As a result of these investigations, a comparison was made between the effect of humus from raw peat and that from bacterised peat and its various fractions, upon the nitrogen-fixing organisms in liquid culture.

It has already been shown by Krzemieniewski† that the addition of natural humus to the culture medium in which *Azotobacter chroococcum* is growing results in a greatly increased nitrogen fixation, so a solution of this natural humus was prepared by treating some of the raw peat used in the preparation of the bacterised peat with just sufficient sodium carbonate solution to

* Bottomley, W. B., 'Roy. Soc. Proc.,' B, vol. 88, pp. 237–247 (1914).
† Krzemieniewski, 'Bull. Acad. Sci. Cracovie,' No. 9, pp. 929–1050 (1908).

extract all the brown soluble matter. Part of this crude sodium humate was set aside for use in the culture media. The remainder was acidified with dilute hydrochloric acid, the precipitate filtered off, washed thoroughly, and redissolved in just sufficient sodium carbonate solution. Part of this purer sodium humate was preserved for experiment, and part was treated with a little calcium chloride solution, the precipitate of calcium humate formed being thoroughly washed. A crude ammonium humate was also prepared by extracting raw peat with a slight excess of ammonium hydrate solution, filtering the extract, and removing the excess of ammonia by evaporation on the water-bath. A solution of artificial sodium humate was obtained by dissolving artificial humic acid, prepared by boiling sucrose with dilute hydrochloric acid in the usual way, in the requisite amount of sodium carbonate solution. All of these preparations were neutral when added to the culture media.

A pure culture of *Azotobacter chroococcum* was isolated from soil and cultivated in a solution consisting of 100 c.c. distilled water, 1 grm. mannite, 0·2 grm. K_2HPO_4, 0·02 grm. $MgSO_4$, and 0·2 grm. $CaCO_3$. A number of flasks each containing 100 c.c. of this medium were divided into series of six each. To the various series the additions shown in the Table below were made, and all were inoculated with 1 c.c. of a uniform suspension of *Azotobacter*. Two flasks of each series were then sterilised in an autoclave at 135° C. to serve as controls, and the whole set was incubated for ten days at 26° C. Analysis by the Kjeldahl method for the nitrogen content then gave the following results:—

Table IV.

Flask.	Contents of each flask in each series.	Nitrogen content after 10 days.		Gain in nitrogen.	
		mgrm.		mgrm.	
1	Control	0·4	0·4		
2	"	0·4			
3	100 c.c. mannite solution	5·6		5·2	5·0
4	" " "	5·4		5·0	
5	" " "	5·3		4·9	
6	" " "	5·4		5·0	
7	Control	2·4	2·5		
8	"	2·6			
9	100 c.c. mannite solution + crude sodium humate from 0·25 grm. peat	11·1		8·6	8·8
10	" " " "	11·5		9·0	
11	" " " "	11·4		8·9	
12	" " " "	11·7		9·2	

Table IV—*continued.*

Flask.	Contents of each flask in each series.	Nitrogen content after 10 days.	Gain in nitrogen.
		mgrm.	mgrm.
13	Control	1·8 } 1·9	
14	"	2·0	
15	100 c.c. mannite solution + purer sodium humate from 0·25 grm. peat	7·3	5·4 } 5·2
16	" " " "	6·9	5·0
17	" " " "	7·4	5·5
18	" " " "	7·0	5·1
19	Control	6·4 } 6·4	
20	"	6·4	
21	100 c.c. mannite solution + crude ammonium humate from 0·25 grm. peat	15·0	8·6 } 8·3
22	" " " "	14·8	8·4
23	" " " "	14·4	8·0
24	" " " "	14·5	8·1
25	Control	1·6 } 1·6	
26	"	1·6	
27	100 c.c. mannite solution + purer calcium humate from 0·25 grm. peat	6·5	4·9 } 5·0
28	" " " "	6·2	4·6
29	" " " "	6·6	5·0
30	" " " "	7·0	5·4
31	Control	0·7 } 0·6	
32	"	0·5	
33	100 c.c. mannite solution + artificial sodium humate from 0·1 grm. artificial humic acid	5·9	5·3 } 5·1
34	" " " "	6·2	5·6
35	" " " "	5·6	5·0
36	" " " "	5·3	4·7
37	Control	5·1 } 5·1	
38	"	5·1	
39	100 c.c. mannite solution + water extract of 0·25 grm. bacterised peat (sterilised)	18·9	13·8 } 14·1
40	" " " "	19·7	14·6
41	" " " "	19·2	14·1
42	" " " "	19·0	13·9
43	Control	2·0 } 2·1	
44	"	2·2	
45	100 c.c. mannite solution + water extract of 0·25 grm. raw peat	5·5	3·4 } 3·6
46	" " " "	6·1	4·0
47	" " " "	5·6	3·5
48	" " " "	5·8	3·7

It is apparent from these figures that the crude humus extracted from the raw peat by alkalies has the effect of increasing to some extent the nitrogen fixation of *Azotobacter* in culture solution, and that this property is not retained when the humus is further purified. Remy and Rösing* obtained

* Remy and Rösing, 'Centr. Bakt. Par.,' Abt. II, vol. 30, pp. 349–384 (1911).

comparable results, but they attributed the beneficial effect of the humus solely to the iron which it contains. In view of this statement, it must be pointed out that both the purified sodium humate and the calcium humate used in the above experiments contained appreciable quantities of iron, which was presumably adsorbed by the colloidal humus, and was by no means entirely removed by the subsequent solution and re-precipitation. Further, while the sodium humate must contain quite as much iron as does the water extract of bacterised peat, since both have been prepared from the same kind of raw peat, yet the effect of the bacterised peat is markedly greater than that of the chemical preparations from raw peat. The inference seems to be that during the bacterisation some soluble organic substances are produced, besides the soluble humates, which have the effect of greatly increasing the nitrogen fixation by *Azotobacter*. These substances appear to be formed in comparatively small quantities during treatment of the peat with weak alkalies, since such a preparation increases fixation to some extent, but they also appear to be lost during further purification of the crude humate. Such substances are not present in raw peat, at least in a water-soluble condition, since a water extract of such peat depresses the rate of nitrogen fixation. That the beneficial effect of the crude humus is not due to any physical action of the colloidal extract is shown by the results with artificial humus, which appears to have practically no effect. This is in accordance with the results of Krzemieniewski.*

The addition of natural humus to the culture medium of *Azotobacter*, in the hands of practically all investigators, has proved to be beneficial to nitrogen fixation, but the degree of benefit obtained differs very widely, rising in Krzemieniewski's* researches from 2·32 mgrm. nitrogen fixed without humus to 21·52 mgrm. with sodium humate from soil. This divergence is very probably due to the varying degree of bacterial decomposition which has taken place in the humus before extraction; the greater the decomposition, the better being the result; and it is most probable that in bacterised peat the bacterial action has taken place under the circumstances most favourable for the production of the essential organic substances. This conclusion receives support from the work of Löhnis and Green†, who found that the humus from fresh stable manure increased fixation by 9·8 mgrm., while that from similar manure which had been "humified" resulted in an increase of 14·4 mgrm. A similar experiment, which they carried out with peat, showed no difference between the effect of fresh and

* Krzemieniewski, 'Bull. Acad. Sci. Cracovie,' No. 9, pp. 929–1050 (1908).

† Löhnis and Green, 'Centr. Bakt. Par.,' Abt. II, vol. 40, pp. 52–60 (1914).

humified peat, evidently owing to the fact that they simply mixed the materials with sand, and allowed them to remain thus for 4½ months, depending solely upon the bacteria which they contained for the "humifying" process. Stable manure is teeming with such organisms, and conditions were ideal for their further action, but peat is practically devoid of them, and they must be added under suitable conditions before decomposition can take place. Hence the difference in the results obtained.

In order to investigate further the effect of these organic substances in bacterised peat, an alcoholic extract, and also the phosphotungstic and silver fractions, were employed. In all cases where an alcoholic extract was used, the alcohol was driven off at a low temperature by means of a fan before use, and the residue taken up in distilled water. Where a water extract was employed, this was sterilised in an autoclave at 135° C., in order to kill off any bacteria already present.

As already shown by Bottomley, the addition of the alcoholic extract of 1 grm. of bacterised peat to every 100 c.c. of culture solution resulted in a marked increase in the rate of nitrogen fixation, so a comparison was made of the action of the different fractions of the peat. A set of 30 flasks was prepared, all containing 100 c.c. of the mannite solution, and divided into five series of six flasks each. To the various series were made the additions described below, and all the flasks were inoculated with 1 c.c. of a suspension of a crude culture of *Azotobacter* from soil. A similar set of 30 flasks was also prepared and inoculated with a pure culture. Two flasks of each series were sterilised for controls, and all were incubated for 10 days, when upon analysis the results set out in Table V were obtained.

Throughout the experiments, the beneficial effect of the fractions from bacterised peat was manifested by the fact that, after about three days, while the cultures in mannite alone showed still only a faint cloudiness, a definite scum was already produced on the surface of those containing these extracts. The figures obtained show a progressive superiority in the effect of the phosphotungstic, silver, alcoholic and water extracts, and, while they point to the conclusion that the active substances are not separated quantitatively by the methods so far adopted, yet it is obvious that all the auximone fractions have the power of increasing the rate of nitrogen fixation of *Azotobacter*. It should be noted that none of the alcoholic, phosphotungstic, or silver fractions contained any trace of iron.

The effect of the auximone fractions upon *Bacillus radicicola*, the nitrogen-fixing organism of the leguminous nodules, was then investigated. Pure cultures were obtained from the nodules of various leguminous plants, notably, broad bean, sweet pea, lucerne, clover and hop trefoil, and the

Table V.

Flask.	Contents of each flask in each series.	Nitrogen content after 10 days.				Gain in nitrogen in 10 days.			
		Crude.		Pure.		Crude.		Pure.	
		mgrm.		mgrm.		mgrm.		mgrm.	
1	Control	0·2	0·2	0·1	0·1				
2	,,	0·2		0·1					
3	100 c.c. mannite solution	6·5		4·1		6·3		4·0	
4	,, ,, ,,	6·1		4·3		5·9	6·0	4·2	4·3
5	,, ,, ,,	6·2		4·4		6·0		4·3	
6	,, ,, ,,	5·9		4·7		5·7		4·6	
7	Control	10·4	10·5	10·3	10·3				
8	,,	10·6		10·3					
9	100 c.c. mannite solution + water extract of 0·5 grm. bacterised peat	24·8		26·3		14·3		16·0	
10	,, ,, ,,	25·5		25·6		15·0	14·5	15·3	15·8
11	,, ,, ,,	25·1		26·2		14·6		15·9	
12	,, ,, ,,	24·7		26·3		14·2		16·0	
13	Control	2·1	2·2	2·2	2·1				
14	,,	2·3		2·0					
15	100 c.c. mannite solution + alcoholic extract of 1 grm. bacterised peat	16·1		17·9		13·9		15·8	
16	,, ,, ,,	16·4		17·7		14·2	14·1	15·6	15·6
17	,, ,, ,,	16·6		18·1		14·4		16·0	
18	,, ,, ,,	16·0		17·3		13·8		15·2	
19	Control	0·4	0·4	0·3	0·3				
20	,,	0·4		0·3					
21	100 c.c. mannite solution + phosphotungstic fraction of 1 grm. bacterised peat	10·6		10·1		10·2		9·8	
22	,, ,, ,,	10·3		9·9		9·9	10·2	9·6	9·6
23	,, ,, ,,	10·7		9·5		10·3		9·2	
24	,, ,, ,,	10·8		10·2		10·4		9·9	
25	Control	0·2	0·2	0·2	0·2				
26	,,	0·2		0·2					
27	100 c.c. mannite solution + silver fraction of 1 grm. bacterised peat	10·8		10·0		10·6		9·8	
28	,, ,, ,,	11·0		10·2		10·8	10·4	10·0	10·0
29	,, ,, ,,	10·1		10·3		9·9		10·1	
30	,, ,, ,,	10·5		10·4		10·3		10·2	

effect of the auximones on all these varieties was tested by cultivating them in a solution consisting of 100 c.c. distilled water, 1 grm. sucrose, 0·2 grm. K_2HPO_4, 0·02 grm. $MgSO_4$, and 0·2 grm. $CaCO_3$. Similar results to those shown with *Azotobacter* were obtained with every variety of the organism tested. The following mean fixations obtained with broad bean

organisms, from six series each consisting of six flasks, are typical of the results with *Bacillus radicicola* :—

		Mgrm.
Sucrose solution		2·6
" "	+ water extract bacterised peat	7·7
" "	+ alcoholic extract bacterised peat	6·5
" "	+ phosphotungstic fraction bacterised peat	5·6
" "	+ silver fraction bacterised peat	6·0
" "	+ water extract raw peat	1·9

The difference in the density of the cultures containing the fractions from bacterised peat, and those containing only sucrose, or sucrose with raw peat, was very marked throughout the experiments, and afforded a very sure indication of the greater activity of the organisms in those cultures containing the auximone fractions.

Both from these experiments and from those with *Azotobacter*, it is evident that certain organic substances, the nature of which has not yet been investigated, can be separated from the prepared peat, and that these substances have an appreciable effect upon the rate of nitrogen fixation by these organisms.

Nitrification.

Experiments on the second group of bacteria concerned in the nitrogen cycle, that is, the nitrifying organisms, were also carried out in soil and in liquid culture. Ever since the isolation, in purely inorganic media, by Winogradsky,* of the particular organisms concerned, the effect of organic matter upon the rate of nitrification has provoked a considerable amount of discussion. Winogradsky* himself stated that the presence of nitrogenous organic matter is inhibitory to the organisms, but further investigation by Müntz and Lainé† revealed the fact that humus in soil has no deleterious effect upon the process. Very soon Coleman‡ showed that organic matter is injurious only in culture solutions, and subsequent research by Stevens and Withers§ fully confirmed this statement, and proved that in soils the presence of organic matter may even help the process. Karpinski and Niklewski‖ have further stated that the presence of small amounts of some

* Winogradsky, 'Ann. de l'Inst. Pasteur,' vol. 4, 1. Mem., pp. 213–231; 2. Mem., pp. 257–275; 3. Mem., pp. 760–771 (1890).

† Müntz and Lainé, 'Compt. Rend.,' vol. 142, pp. 430–435 (1906).

‡ Coleman, 'Centr. Bakt. Par.,' Abt. II, vol. 20, pp. 401–420 (1908).

§ Stevens and Withers, 'Centr. Bakt. Par.,' Abt. II, vol. 27, pp. 169–186 (1910).

‖ Karpinski and Niklewski, 'Bull. Acad. Sci. Cracovie,' 1907, pp. 596–615.

organic substances is favourable to nitrification in impure culture, and that especially good effects are produced by humates in very small amounts.

In order to examine the effect of bacterised peat upon nitrification in soil a preliminary experiment was carried out upon a rich garden soil from Kew. Four portions of soil, each weighing 24 oz., were taken, and each of two of them was mixed with one part in ten by volume of bacterised peat. Two portions of another soil were also taken, one being treated and the other serving as control. Three samples of each were weighed out for nitrate determination, and the six soils were kept in wide-mouthed glass bottles at about 20° C. as before. They were aërated daily, and the moisture content kept as uniform as possible. At intervals they were all examined for nitrate, the determinations being made by the phenol-sulphonic acid method. The figures obtained by this method were very concordant, and at the outset a comparison was made between the results thus obtained and those given by the method of reduction by sodium amalgam, the ammonia being separately determined when the latter process was adopted. A close agreement was shown between the figures, and the more cumbersome sodium amalgam method was therefore discarded.

When dealing with solutions containing the water extract of the peat, the deep colour could readily be discharged by precipitating the humic acid with a drop of dilute hydrochloric acid and shaking the filtrate with carbon black (D. Elf brand). The colourless liquid thus obtained was then analysed, and concordant results obtained by this method of procedure.

The figures obtained at intervals from the above soils were:—

Table VI.

	Nitrate content (parts per million of nitric nitrogen—mean of three determinations).				
	Originally.	After 7 days.	14 days.	21 days.	28 days.
1. Soil A alone	30	43	57	107	208
2. " "	31	45	56	112	200
3. Soil B alone	35	46	81	95	99
4. Soil A + bacterised peat	27	87	450	540	539
5. " " "	29	91	489	528	541
6. Soil B + bacterised peat	30	156	248	319	372

Each of the figures in the above and following Tables of soil analyses represents the mean of three determinations, the maximum deviation from the mean being ±5.

The fact that the increase in nitrate content in the soils A containing

bacterised peat in the above Table had practically ceased during the last week, suggested that either the nitrate had accumulated to such an extent that it had become inhibitory to the organisms concerned, or that the whole of the available nitrogen had been nitrified.

In order to test this, each of the two soils containing bacterised peat was placed on a filter paper in a large Buchner funnel fitted into a vacuum flask. The soils were covered with distilled water, and the flasks exhausted as rapidly as possible by means of a pump. They were then dried down to a suitable moisture content as rapidly as possible at a low temperature by means of a fan, and were re-incubated after samples had been taken for analysis. By this time two days had elapsed since the sampling on the 28th day, and during this time the control soils had been incubated as usual. These soils were not re-examined on the 30th day, but in the Table below the figures are given as for the 28th day. The subsequent results obtained were:—

Table VII.

	Nitrate content (parts per million—mean of three determinations).			
	After 30 days.	35 days.	42 days.	49 days.
1. Soil alone	208	222	234	177
2. ,, ,,	200	230	179	175
3. Soil + bacterised peat	204	230	298	337
4. ,, ,, ,,	189	199	310	350

These results indicated that the addition of bacterised peat to a fertile soil results in a rapid increase in nitrate content up to a maximum, when the concentration of nitrate becomes inhibitory to the growth of the organisms, and nitrification ceases. If this accumulation be partially removed by rapid washing, nitrification again proceeds.

As in the case of nitrogen fixation, nitrification is essentially an aërobic process, and may have been facilitated by the better aëration of the soil, so a comparison was made of the effect of introducing similar bulky matter in the form of raw peat and stable manure. An examination was also made of the effect of chemically produced soluble humate in the form of peat which had been treated with 2 per cent. of its weight of sodium carbonate. The soil chosen for this experiment was a fertile soil from Chelsea Physic Garden, and the organic manures were added in the proportion of one part in ten by bulk. The soils containing raw peat also received a dressing of 1 per cent. of powdered chalk, in order to counteract as far as possible any inhibitory

effect due to the natural acidity of the peat. The various soils were incubated precisely as before, and at the end of 28 days it was found that the nitrate-content of the soil containing bacterised peat had approached very near to the maximum quantity shown in the previous experiment. These two samples of soil were accordingly leached with distilled water, as described above, to remove the accumulations of nitrate. The nitric nitrogen which they contained was then estimated before they were re-incubated, and the figures obtained are shown in the following Table :—

Table VIII.

	Nitrate content (parts per million—mean of three determinations).						
	Originally.	After 7 days.	21 days.	28 days.	35 days.	47 days.	54 days.
1. Soil alone	70	72	86	92	105	136	156
2. " "	67	68	86	95	112	140	163
3. Soil + raw peat	66	73	77	84	79	81	59
4. " "	64	76	80	89	80	80	66
5. Soil + carbonated peat	61	81	98	108	136	143	173
6. " " "	67	84	92	99	142	167	186
7. Soil + bacterised peat	59	90	356	460 leached = 37	94	158	195
8. " " "	60	110	380	450 leached = 62	101	174	213
9. Soil + stable manure ...	65	65	71	78	89	101	128
10. " " "	60	64	80	85	89	109	116

It is evident from the results so far given that the addition of bacterised peat to the soil results in an enormous accumulation of nitrate, which would naturally be removed fairly rapidly by any growing crops. The figures in the above Table also indicate that the introduction of the chemically produced humate results in a slight increase in nitrate production, while the addition of opening material in the form of raw peat and stable manure tends rather to depreciate than increase the rate of nitrification in the soil. The causes of this depreciation were not further investigated, since it had no particular bearing upon the work.

There is one other important factor which must be taken into consideration in connection with the rapid nitrification in soils containing bacterised peat. It is well known that this material contains a certain amount of soluble nitrogen in the form of ammonium humate, and the question arises as to whether this ammonia is merely being nitrified at a normal rate, or whether any stimulation of the soil organisms is taking place in addition.

In order to put this to the test, an estimation was made of the soluble nitrogen in bacterised peat, and a comparison was then made between the rate of nitrification in soil containing one-tenth of its volume of this material and in that containing an amount of ammonia equivalent to the soluble nitrogen thus introduced, in the form of ammonium sulphate. Portions consisting of 800 grm. of a fresh sample of Chelsea soil were used, and one-tenth of the volume of this of bacterised peat weighed 32 grm., containing 40 per cent. of moisture, the actual dry weight of the material introduced into the soil being thus equal to 19·2 grm. This contained 1·8 per cent. of soluble nitrogen as ammonia, so that the soluble ammonia introduced was equivalent to 1·63 grm. ammonium sulphate, and this addition was made to each of two portions of soil. All the soils were incubated just as before, and the results obtained were :—

Table IX.

	Nitrate content (parts per million—mean of three determinations).				
	Originally.	After 7 days.	14 days.	35 days.	49 days.
1. Soil alone	25	28	42	78	104
2. " "	27	29	40	82	110
3. Soil + $(NH_4)_2SO_4$	26	45	67	195	276
4. " "	25	49	75	190	259
5. Soil + bacterised peat	20	54	210	480	524
6. " " "	22	61	201	468	541

It is evident that the rate of nitrification in soil containing bacterised peat is greater than that in soil containing an equivalent quantity of soluble ammonia in the form considered most suitable for nitrification; so that it appears probable that, apart from supplying nitrifiable nitrogen, the addition of bacterised peat increases the activities of the soil organisms. That there is some factor in this material which has a beneficial effect upon nitrification in soils, apart from any nitrogen it contains, is shown by the effect of the phosphotungstic fraction upon nitrate formation. Attention has already been drawn to this effect by Bottomley,* but fuller experiments have since been made. To various uniform samples of the same soil were added the phosphotungstic fraction obtained from amounts of bacterised peat equivalent to (*a*) one-tenth the volume of soil, (*b*) one-fifth the volume of soil, (*c*) one-sixteenth the volume of soil, and (*d*) one-eighth the volume of soil

* Bottomley, W. B., 'Roy. Soc. Proc.,' B, vol. 89, pp. 102–108 (1916).

respectively. The proportion of one part of bacterised peat in ten of soil is that used practically throughout the experiment, but it was anticipated that, since in all probability by no means the whole of the active substance is separated by the phosphotungstic acid method, an addition of this fraction equivalent to one part of peat in ten might possibly be too small to produce the maximum effect. For this reason, experiments were made with the phosphotungstic fraction from double the amount of peat normally used, and also from proportions of one-eighth and one-sixteenth the volume of soil. The soils, incubated and sampled from time to time, gave the following results:—

Table X.

	Nitrate content (parts per million—mean of three determinations).			
	Originally.	After 15 days.	29 days.	40 days.
1. Soil alone	31	50	88	112
2. " "	35	48	96	120
3. Soil + phosphotungstic fraction from 1 in 10	36	84	220	320
4. " " " " 1 in 10	33	90	211	309
5. " " " " 1 in 5	34	112	304	462
6. " " " " 1 in 5	36	105	302	448
7. " " " " 1 in 8	35	83	232	346
8. " " " " 1 in 8	31	85	241	335
9. " " " " 1 in 16	32	40	130	198
10. " " " " 1 in 16	31	53	141	205

The addition, in sufficient quantity, of the phosphotungstic fraction thus appears to increase the rate of nitrification in soils. It has, however, been shown above that such addition also increases the nitrogen fixation, and the possibility arises that the increase in nitrate content may be due to the nitrification of the nitrogen fixed rather than to direct action upon the nitrifying organisms themselves.

This point could only be elucidated by examining the effect of the auximone fractions upon nitrification in liquid culture. Accordingly, a crude nitrifying culture was obtained by inoculating some good garden soil into Erlenmeyer flasks, each containing 100 c.c. of the following medium:—Tap water, 100 c.c.; ammonium sulphate, 0·1 grm.; dipotassium phosphate, 0·1 grm.; basic magnesium carbonate, 0·2 grm.; and incubating at 26° C. for seven days. This was sub-cultured three times before any attempt was made to experiment with the auximone fractions, but then it was found that the addition of the phosphotungstic or silver fraction to the culture resulted in the formation of the thick scum of some foreign organisms already described

by Bottomley,* and no nitrate was formed in the medium. It was only after long-continued sub-culturing that a nitrifying culture was obtained free from these organisms, and the experiments recorded were carried out with this comparatively pure culture.

A preliminary experiment showed that the water extract of bacterised peat is directly nitrifiable, hence the effect of this extract upon the rate of nitrification in normal nitrifying solution cannot be tested. An experiment was therefore made to determine whether the auximone fractions had any effect upon the rate of nitrification in ammonium sulphate solution. Portions of 100 c.c. of Winogradsky's medium, described above, were put into each of 24 flasks, divided into four series of six each, with the additions shown in the Table below. When all had been inoculated, and two of each series sterilised for controls, the whole set was incubated at 26° C. for seven days. The nitrate content of 25 c.c. of each was then estimated as before, with the following results :—

Table XI.

Flask.	Contents.	Nitric nitrogen in 25 c.c.	Nitric nitrogen in whole.
		mgrm.	mgrm.
1	Control	0·01	0·04 } 0·038
2	"	0·009	0·036
3	100 c.c. Winogradsky's medium	0·18	0·72 } 0·77
4	" " "	0·21	0·84
5	" " "	0·20	0·80
6	" " "	0·18	0·72
7	Control	0·01	0·04 } 0·04
8	"	0·01	0·04
9	100 c.c. Winogradsky's medium + alcoholic extract of 1 grm. bacterised peat	0·31	1·24 } 1·16
10	" " " "	0·30	1·20
11	" " " "	0·27	1·08
12	" " " "	0·28	1·12
13	Control	0·009	0·036 } 0·036
14	"	0·009	0·036
15	100 c.c. Winogradsky's medium + phosphotungstic fraction of 1 grm. bacterised peat	0·27	1·08 } 1·14
16	" " " "	0·31	1·24
17	" " " "	0·27	1·08
18	" " " "	0·29	1·16
19	Control	0·01	0·04 } 0·04
20	"	0·01	0·04
21	100 c.c. Winogradsky's medium + silver fraction of 1 grm. bacterised peat	0·34	1·36 } 1·44
22	" " " "	0·36	1·44
23	" " " "	0·39	1·56
24	" " " "	0·35	1·40

* Bottomley, W. B., 'Roy. Soc. Proc.,' B, vol. 89, pp. 102–108 (1915).

It is apparent from these figures that the auximone fractions have the power of increasing the rate of nitrification in culture solution. If organic matter in general has the effect of depressing nitrification, the additions made in these fractions are evidently too slight to produce any such result. However, since the water extract of bacterised peat, a highly organic solution, nitrifies so readily, an experiment was made to compare the rate of nitrification in Winogradsky's solution with that in bacterised peat extract containing an equivalent quantity of nitrogen, and also with nitrification in a solution of ammonium humate containing an equal quantity of nitrogen. The humic acid for the ammonium humate was extracted from raw peat in the usual manner, purified by re-dissolving in ammonium hydrate, and re-precipitating with hydrochloric acid twice over, finally dissolving up in excess of ammonia, and then expelling the excess by evaporation on the water-bath. The ammonium humate thus obtained was dissolved in distilled water and the ammonia in an aliquot part determined. The concentrated solution was then diluted until it contained the same proportion of ammonia as Winogradsky's medium. The requisite amounts of potassium phosphate and magnesium carbonate were added to the flasks containing this solution. The bacterised peat extract was prepared by making a concentrated extract, determining the nitrogen it contained, and diluting until it contained the same proportion as Winogradsky's medium and the ammonium humate, the phosphate and base being added as before. A series of 30 flasks was prepared, as shown in the Table below, and after inoculation and incubation for seven days 25 c.c. from each flask was analysed for nitric nitrogen. The whole set was then re-incubated for a further period of seven days, when 10 c.c. of each was again examined. The figures obtained are given in Table XII (p. 526).

Although the greater part of the nitrogen in the water extract of bacterised peat occurs in the form of ammonium humate, it appears from the above figures that this substance, in the pure condition, is comparatively slowly nitrifiable, and even the addition of auximones, which increases the rate of nitrification of the ammonium sulphate to an appreciable extent, does not render that of ammonium humate equal to that of ammonium sulphate alone. This is probably due to the depressing effect of the organic matter, although the readily nitrifiable bacterised peat extract contains a similar quantity. It follows that, in this extract, there must be some other factor to be taken into consideration besides the ammonia content and the auximone fraction thus far isolated.

It appears most probable, from all the results obtained, that the methods

employed fail to extract the total amount of the activating substances present in the bacterised peat, although they show that the material separated in the various fractions has the effect of appreciably increasing the rate of nitrification as well as nitrogen fixation.

Table XII.

Flask.	Contents.	7 days.			14 days.		
		Nitric nitrogen in 25 c.c.	Equivalent to nitric nitrogen in whole.		Nitric nitrogen in 10 c.c.	Equivalent to nitric nitrogen in whole.	
		mgrm.	mgrm.		mgrm.	mgrm.	
1	Control	0·01	0·04	0·04	0·005	0·05	0·05
2	"	0·01	0·04		0·005	0·05	
3	100 c.c. Winogradsky's medium	0·18	0·72		0·51	5·1	
4	" " "	0·19	0·76	0·72	0·55	5·5	5·3
5	" " "	0·18	0·72		0·54	5·4	
6	" " "	0·17	0·68		0·52	5·2	
7	Control	0·01	0·04	0·04	0·004	0·04	0·045
8	"	0·01	0·04		0·005	0·05	
9	100 c.c. Winogradsky's medium + silver fraction from 1 grm. bacterised peat	0·31	1·24		0·75	7·5	
10	" " "	0·30	1·20	1·17	0·77	7·7	7·6
11	" " "	0·28	1·12		0·79	7·9	
12	" " "	0·28	1·12		0·73	7·3	
13	Control	0·006	0·024	0·028	0·003	0·03	0·03
14	"	0·008	0·032		0·003	0·03	
15	100 c.c. ammonium humate solution	0·11	0·44		0·25	2·5	
16	" " "	0·12	0·48	0·45	0·22	2·2	2·4
17	" " "	0·12	0·48		0·22	2·2	
18	" " "	0·10	0·40		0·26	2·6	
19	Control	0·006	0·024	0·024	0·003	0·03	0·025
20	"	0·006	0·024		0·002	0·02	
21	100 c.c. ammonium humate solution + silver fraction from 1 grm. bacterised peat	0·16	0·64		0·37	3·7	
22	" " "	0·15	0·60	0·65	0·35	3·5	3·4
23	" " "	0·18	0·72		0·36	3·6	
24	" " "	0·16	0·64		0·30	3·0	
25	Control	0·016	0·064	0·056	0·007	0·07	0·06
26	"	0·012	0·048		0·005	0·05	
27	100 c.c. bacterised peat extract	0·35	1·40		0·79	7·9	
28	" " "	0·35	1·40	1·42	0·81	8·1	7·8
29	" " "	0·38	1·52		0·78	7·8	
30	" " "	0·34	1·36		0·76	7·6	

Ammonification.

The cycle of changes which nitrogenous substances undergo in the soil is a complex one, and in marked contrast to the two processes investigated above

are the two chief decomposition processes, ammonification and denitrification. These result in the breaking down of the soil organic matter and nitrates, with the liberation in the form of ammonia and free gaseous nitrogen of the element which has been "fixed" and oxidised in the two processes of nitrogen fixation and nitrification. As these two decomposition processes involve reactions which are directly opposed to those concerned in the two already considered, it was thought possible that an investigation of the effect of auximones upon the bacteria concerned in them would give some indication as to whether the auximones merely stimulate all classes of bacteria equally, or whether they play some definite part in the building up of the nitrogenous molecule.

It was practically impossible to carry out experiments on ammonification in a soil which had been mixed with bacterised peat, in the same way as had been done for nitrogen fixation and nitrification, on account of the ammonia content of the bacterised peat and the rapid nitrate formation and other changes in the nitrogen compounds in such a mixture. The only alternative was to depend upon the results obtained in liquid culture, and here again, on account of the ammonia contained in the water extract of bacterised peat, it became difficult to test the effect of this extract upon the process of ammonification. It appears, however, from the results obtained in the experiments recorded above, that the auximone fractions are largely responsible for the increased activity of the bacteria hitherto investigated; hence tests were made of the effect upon the ammonifying organisms of the alcoholic, phosphotungstic and silver fractions alone.

An investigation was first made of the influence of the addition of these fractions upon the "ammonifying" or "putrefactive" power of the soils used in the previous experiments, this putrefactive power being determined by the method in general use described by Remy.*

Sixteen flasks were prepared, each containing 100 c.c. of 1 per cent. peptone solution and 10 grm. of fine air-dried Chelsea soil. To four of these was added the alcoholic extract, to another four the phosphotungstic fraction, and to a third four the silver fraction, of 1 grm. of bacterised peat. The whole set was incubated at 22° C. for five days, then 2 grm. of calcined magnesia and a few drops of paraffin (to prevent frothing) were added to each. The contents of each flask were distilled, and the distillate received in decinormal sulphuric acid, which was then titrated with decinormal sodium hydrate solution. The figures obtained were:—

* Remy, 'Centr. Bakt. Par.,' Abt. II, vol. 8, pp. 657–662 (1902).

Table XIII.

Flask.	Contents.	Acid neutralised.	NH₃ present.	
		c.c.	mgrm.	
1	Soil + 1 per cent. peptone	35·0	59·50	60·60
2	" " "	36·1	61·37	
3	" " "	35·5	60·35	
4	" " "	36·0	61·20	
5	Soil + 1 per cent. peptone + alcoholic extract of 1 grm. bacterised peat	35·9	61·08	61·11
6	" " " "	35·7	60·69	
7	" " " "	36·0	61·20	
8	" " " "	36·2	61·54	
9	Soil + 1 per cent. peptone + phosphotungstic fraction from 1 grm. bacterised peat	36·2	61·54	61·16
10	" " " "	36·3	61·71	
11	" " " "	35·8	60·86	
12	" " " "	35·6	60·52	
13	Soil + 1 per cent. peptone + silver fraction from 1 grm. bacterised peat	36·1	61·37	60·94
14	" " " "	35·8	60·86	
15	" " " "	35·6	60·52	
16	" " " "	35·9	61·03	

An identical series of experiments was carried out with each of the soils obtained from Kew, and these yielded similar results, all failing to show any effect of the auximones, stimulating or otherwise, upon the ammonifying power of the soils. In view of these results, an examination was made of the influence of these substances upon the ammoniacal fermentation of urea.

For this purpose, a mixed culture of ammonifying organisms was obtained from rotting manure by inoculating the latter into a culture medium consisting of Witte's peptone 1 grm., urea 10 grm., Lemco 5 grm., distilled water 100 c.c., the whole being neutralised with ammonium carbonate solution. A drop of this mixed culture was then inoculated into a solution of urea 50 grm., mono-potassium phosphate 25 grm., sodium acetate 10 grm., distilled water 1000 c.c., this solution also being just neutralised with ammonium carbonate solution. The culture thus obtained was sub-cultured three successive times into flasks of the same medium, three days' incubation elapsing between each sub-culture. Sixteen flasks, each containing 100 c.c. of the same medium, with the additions shown in the Table below, were then inoculated each with 1 c.c. of the culture of urea-splitting organisms thus obtained. The whole set was incubated at 22° C., 10 c.c. of each being withdrawn after periods of 24 and 48 hours respectively, and titrated with decinormal sulphuric acid, methyl orange being used as indicator. The figures obtained were as follows:—

Table XIV.

Flask.	Contents.	In 24 hours, 10 c.c. required		In 48 hours, 10 c.c. required		
		c.c. acid	= mgrm. NH_3.	c.c. acid	= mgrm. NH_3.	
1	100 c.c. ammonifying culture	22 ·5	38 ·25	22 ·2	37 ·74	38 ·33
2	" " "	22 ·0	37 ·40	23 ·0	39 ·10	
3	" " "	22 ·4	38 ·18	22 ·5	38 ·25	
4	" " "	22 ·5	38 ·25	22 ·5	38 ·25	
5	100 c.c. ammonifying culture + alcoholic extract of 1 grm. bacterised peat	21 ·8	37 ·06	22 ·3	37 ·91	38 ·13
6	" " "	22 ·4	38 ·18	22 ·4	38 ·18	
7	" " "	22 ·5	38 ·25	22 ·5	38 ·25	
8	" " "	22 ·0	37 ·40	22 ·4	38 ·18	
9	100 c.c. ammonifying culture + phosphotungstic fraction of 1 grm. bacterised peat	21 ·8	37 ·06	22 ·5	38 ·25	38 ·33
10	" " "	22 ·0	37 ·40	22 ·8	38 ·76	
11	" " "	22 ·0	37 ·40	22 ·3	37 ·91	
12	" " "	22 ·3	37 ·91	22 ·6	38 ·42	
13	100 c.c. ammonifying culture + silver fraction of 1 grm. bacterised peat	21 ·8	37 ·06	22 ·4	38 ·18	37 ·83
14	" " "	22 ·2	37 ·74	22 ·4	38 ·18	
15	" " "	21 ·9	37 ·23	21 ·6	36 ·72	
16	" " "	22 ·0	37 ·40	22 ·5	38 ·25	

These experiments were repeated over and over again, examinations being made of the ammonia produced at the end of periods varying from 6 to 96 hours, but always with similar results. No effect whatever was observed upon the rate of ammonia production, and, in view of the increased activities of the nitrogen-fixing and nitrifying organisms following upon the addition of the auximones to their culture solutions, these results were at first surprising. However, it should be pointed out that these substances are produced in the peat during its "bacterisation," which results in the formation of a certain quantity of ammonium humate, and that they are therefore, at least partially, the products of a bacterial action somewhat similar in nature to the ammonifying process itself. It is scarcely to be expected therefore that the activities of the organisms would be affected by substances bearing a close relation to their own products, unless, as in the case of the nitrifying bacteria, these products had accumulated in such amounts as to bring about an inhibitory effect.

The auximone fractions, however, as has been shown above, have the effect of increasing the rate of nitrification in soil and in culture solution, yet they have no effect upon the rate of ammonification. These results appear to be

directly opposed to the statement by Russell* that "a measure of the speed at which nitrates are formed does not measure the rate of nitrification, but the rate of ammonia production." If this statement that the oxidation of ammonia to nitrates is normally proceeding as fast as ammonia is being formed be true, then the possible sources of nitrifiable nitrogen must be considered, for the auximones themselves introduce only a negligible quantity. There is the possibility that the addition of the auximones may have an effect upon ammonification in soil widely different from that in liquid culture, owing to divergence of conditions; but, apart from this consideration, the fixation of nitrogen introduces an appreciable quantity of this element into the soil, probably in a nitrifiable form. Thus the increase in nitrate content following upon the addition of the auximone fractions to the soil is probably partly accounted for by the nitrification of the element introduced by the nitrogen-fixing organisms, whose activities are also shown to be increased by this addition. Experiments in pure culture, however, show that in the presence of sufficient quantities of nitrifiable nitrogen, the activities of the nitrifying organisms are increased beyond their normal rate by the addition of auximones.

Denitrification.

For the purpose of investigating the effect of the auximone fractions of bacterised peat upon denitrification, methods of liquid culture were again employed. The bacteria which are concerned in this process are responsible for the loss of nitrogen which often follows upon the addition of decomposing organic manures to soil containing nitrates. Since the nitrogen is liberated in the free gaseous form, an estimation of the activity of the organisms can be made approximately by measuring the volume of gas to which they give rise.

For the isolation of these organisms in impure culture, Giltay's solution is most generally employed, but equally good and very uniform results have been obtained during the present work with a medium consisting of calcium tartrate 10 grm., potassium nitrate 10 grm., di-potassium phosphate 0·25 grm., and tap-water 500 c.c. This has the advantage of being simple and very readily made up, so it was used throughout the following experiments.

In order to obtain a culture of the organisms, small portions of decomposing stable manure were introduced into Erlenmeyer flasks of 150 c.c. capacity. The flasks were then filled to the brim with the above medium,

* Russell, E. J., 'Soil Conditions and Plant Growth,' 1915, p. 86.

and each was closed by a well-fitting cork, through which passed a glass tube, reaching almost to the bottom of the flask, and bent at an angle of about 60° just above the cork. The corks were all coated with melted paraffin to render them air-tight. The process of denitrification is an anaërobic one, and the inert gases collected at the top of the flasks, forcing the liquid out through the bent tube and maintaining the anaërobic conditions.

When a crude culture had been obtained in this way, it was sub-cultured four successive times into fresh media before being used for purposes of experiment, and a comparatively pure culture of mixed denitrifying organisms was thus obtained; 1 c.c. of this culture was then transferred to each of the 20 flasks shown in the Table below, and after filling with their respective solutions these flasks were all corked, great care being taken that none of the medium should be spilt, and at the same time that all air bubbles should be excluded.

After 36 hours' incubation at 22° C., the corks were carefully removed, and the volume of gas which had collected in each was measured approximately by filling each flask again with water from a burette.

The results obtained were :—

Table XV.

Flask.	Contents.	Gas formed in 36 hours.	
		c.c.	
1	Culture solution	49	54
2	,, ,,	58	
3	,, ,,	54	
4	,, ,,	55	
5	Culture solution + water extract of 0·5 grm. bacterised peat	54	54
6	,, ,, ,, ,, ,,	56	
7	,, ,, ,, ,, ,,	50	
8	,, ,, ,, ,, ,,	57	
9	Culture solution + alcoholic extract of 1 grm. bacterised peat	4·0	5·2
10	,, ,, ,, ,, ,,	6·0	
11	,, ,, ,, ,, ,,	6·5	
12	,, ,, ,, ,, ,,	4·5	
13	Culture solution + phosphotungstic fraction of 1 grm. bacterised peat	3·0	3·0
14	,, ,, ,, ,, ,, ,,	2·5	
15	,, ,, ,, ,, ,, ,,	4·0	
16	,, ,, ,, ,, ,, ,,	2·5	
17	Culture solution + silver fraction of 1 grm. bacterised peat	29	30
18	,, ,, ,, ,, ,,	27	
19	,, ,, ,, ,, ,,	33	
20	,, ,, ,, ,, ,,	30	

This experiment was repeated several times, always with similar results. At the conclusion of one of these repetitions 1 c.c. of the culture was extracted from each of the flasks and diluted to 100 c.c., and 1 c.c. of each of these was again taken and diluted to 100 c.c. A further similar dilution was made, and then 1 c.c. of each of the 20 equally diluted cultures was inoculated into a sterilised tube of the denitrifying medium, containing 1 per cent. of agar-agar. The inoculation was performed just before the media solidified, the tubes being well shaken to ensure distribution of the organisms. When this was accomplished, they were plugged with cotton wool and incubated at 22° C. The number of bubbles of gas formed in the solid media after a given time indicated the number of colonies in the tubes, and these numbers were practically equal in the tubes inoculated from the pure culture medium and in those from the water extract, very much fewer in number in those from the alcoholic and phosphotungstic fractions, these being again practically equal between themselves, while those from the silver fraction were intermediate in number between these two sets. These numbers bear out the figures obtained above, for it is to be expected that the number of bacteria should be proportional to the activity of the culture.

Similar results having been uniformly obtained in all experiments with a comparatively pure culture, the effect of the addition of the auximones upon the denitrifying power of the soils themselves was examined. The soils from Kew and Chelsea used in the above experiments were investigated, and the culture flasks were prepared precisely as before. Instead of being inoculated with a definite quantity of a pure culture, however, each was inoculated with 5 grm. of a finely sifted uniform sample of the soil to be tested, and was then incubated in the usual manner. The results obtained were concordant throughout with the figures given for the pure culture, a typical set of mean results, obtained with a Chelsea soil, being as follows:—

	Gas formed in 48 hours. c.c.
Culture solution—	
+ soil	63·0
+ soil + water extract 0·5 grm. bacterised peat	64·0
+ soil + alcoholic extract 1 grm. bacterised peat	9·5
+ soil + phosphotungstic fraction 1 grm. bacterised peat	8·1
+ soil + silver fraction 1 grm. bacterised peat	31·0

From the results obtained it appears evident that while the water extract of bacterised peat is practically without effect upon denitrification, the

auximone fractions definitely inhibit the process to a marked degree. Since the auximones are water-soluble, it might be expected that the water extract would also depress the rate of denitrification. However, the presence of some readily oxidisable organic matter is necessary in order that denitrification may proceed, and the medium used in these experiments contains such in the form of tartrate. In addition to this, the water extract supplies extra organic matter, and although the humus of the soil had been found by Stoklasa and Ernest* to be not very serviceable for denitrification, it is quite probable that such an addition might increase the rate of denitrification to a degree sufficient to counterbalance the depressing effect of the auximones contained.

From all the evidence collected in the present work it is apparent that soluble humus, and especially that produced by bacterial decomposition, is a very important factor from the point of view of the activities of soil bacteria. Its effect upon the organisms appears to be largely independent of any inorganic matter which it may contain, or any physical action brought about by its colloidal nature, and is shown to be due to the presence in the humus of growth-promoting substances or auximones. The influence of these auximones upon the organisms concerned in the nitrogen cycle may be briefly summed up in the general statement that they increase the rate of nitrogen fixation and nitrification, depress the rate of denitrification, and do not appreciably affect the rate of ammonification. These results are interesting from the indication they give of the specific rôle of the auximones. If these substances merely act as stimulants to the bacterial protoplasm, it is to be expected that similar effects would be produced by them upon all classes of bacteria. If, on the other hand, they play some definite part in the building up of the complex nitrogenous molecule, it follows that a directly opposite effect might be anticipated from the addition of these substances to two classes of bacteria whose activities are directed upon such widely divergent lines as those concerned in the constructive processes resulting in the oxidation and the fixation of nitrogen in an organic form, on the one hand, and those destructive organisms which bring about its decomposition and liberation in the form of the free element on the other.

In conclusion, I wish to express my sincere thanks to Prof. W. B. Bottomley for the valuable advice and help which he has so kindly given me during the progress of this work.

* Stoklasa and Ernest, 'Centr. Bakt. Par.,' Abt. II, vol. 17, pp. 27–33 (1907).

On the Causes Responsible for the Developmental Progress of the Mammary Glands in the Rabbit during the Latter Part of Pregnancy.

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[PLATE 23.]

The parts played by the various reproductive organs in causing the growth of the mammary gland and the secretion of milk have been the subject of much recent investigation. Most of the work has been done with the rabbit, and the changes which occur in its mammary glands have been studied very minutely.*

The causes of the growth changes in the gland during the first part of pregnancy are now conclusively shown to be due to the influence of the corpus luteum.† These changes in the rabbit culminate at about the 16th day after coitus; after this time in pseudo-pregnant rabbits (*i.e.*, rabbits which have had coitus and developed corpora lutea, but have not become pregnant) the gland undergoes atrophy. If the growth changes of the mammary glands of a series of pregnant and pseudo-pregnant rabbits be compared, it will be seen that the changes are similar until about the 16th day, at which period in the pseudo-pregnant condition the gland begins to atrophy, while in the pregnant animal the gland becomes much thicker, increasing rapidly in weight from the 24th to the 30th day.

The object of the investigations described in the present paper was to determine the causes of this further development of the mammary gland (called by Ancel and Bouin‡ the glandular phase) which takes place after the 16th day of pregnancy.

In the present paper it is shown that, contrary to the commonly accepted opinion, the corpus luteum in the rabbit does not become atrophied during the latter part of pregnancy, but maintains its size till late in gestation and even into the period of lactation, the cause of this development originating in the fœtus. It is also shown that the changes in the mammary gland in the second half of pregnancy are correlated with the further development of the corpora lutea, and consequently that the origin of the

* Schil, 'Recherches sur la Glande Mammaire,' Nancy, 1912.

† Ancel and Bouin, 'Compt. rend. Soc. Biol.,' vol. 66 (1909). For further references see Hammond and Marshall, 'Roy. Soc. Proc.,' B, vol. 87 (1914).

‡ Ancel and Bouin, 'Journ. de Phys. et de Path. générale,' vol. 13 (1911).

influence which causes the growth changes and also the glandular phase of the gland is essentially the same and not different, as Ancel and Bouin have supposed.*

Many methods of research have been used to eliminate the various factors which might possibly cause this development. These are described under the headings of the various tissues or organs which were suspected of being the originators of the stimulus.

The Myometrial Gland.

Ancel and Bouin† state that the myometrial gland occurs in the uterus of the rabbit during the second half of pregnancy. They describe it as consisting of clumps of cells of an epithelioid appearance, lying under the placental cells and between the muscle cells of the circular and longitudinal muscular coats in close proximity to the blood-vessels. They suggest that it is a gland of internal secretion which takes on the functions of the corpus luteum during the second half of pregnancy, controlling the glandular phase of the mammary gland‡ and also the tolerance of the uterus for the fœtus.§

Fraenkel‖ has verified their observations as to the presence of the gland in rabbits. He could not find it, however, in the uteri of many other species of animal examined. On this account, and also on account of its slender vascularity (for a gland of internal secretion), he does not accept their theory as to its function.

Mercier¶ identifies the myometrial gland with cells described by him as nephro-phagocytes, and states that the normal connective tissue of mammals contains these cells, which have the property of phagocytosis, absorbing solid substances and fixing soluble substances injected into the organism. He found that these cells occur only in the pregnant and not in the normal uterus.**

The uteri of many rabbits between the 16th and 30th days of pregnancy have been examined by cutting serial sections across a large number of pieces from each uterus. Epithelioid cells, which correspond to those described by Ancel and Bouin as the myometrial gland, have been found only in one or two cases. In every case they have been found beneath the small yellow patches which are seen in the region of the uterine mucosa

* Ancel and Bouin, 'Journ. de Phys. et de Path. générale,' vol. 13 (1911).

† Ancel and Bouin, 'Compt. rend. Assoc. des Anat.,' 13e. réunion, Paris, 1911.

‡ Ancel and Bouin, 'Compt. rend. Soc. Biol.,' vol. 72 (1912).

§ Ancel and Bouin, 'Compt. rend. de l'Acad. des Sci.,' vol. 154 (1912).

‖ Fraenkel, 'Arch. für Gynaek.,' vol. 99 (1913).

¶ Mercier, 'Compt. rend. Soc. Biol.,' vol. 74 (1913).

** Mercier, 'Compt. rend. Soc. Biol.,' vol. 72 (1912).

opposite the placenta. Since these pustules consist of giant cells (fig. 1) (which are supposed to be detached trophoblast cells) surrounded by a mass of decidual cells, it seems quite possible that the myometrial cells are fœtal cells which have wandered to the muscular coat.

As the cells of the myometrial gland do not occur constantly in all pregnant rabbits, so far as we have been able to investigate, we must conclude that they cannot have the important functions which Ancel and Bouin ascribe to them.

The Maternal Placenta.

Many investigators have attributed to the placenta the property of causing milk secretion (Halban,* Basch†), and, although as a primary cause of the growth changes of the mammary gland in the first half of pregnancy it is shown to be unnecessary, yet it seemed quite possible that it might be responsible for the glandular phase of the second half of pregnancy.

Injection experiments with placental extracts were not tried, since there is great difficulty in getting fresh extracts sterile, and the process of boiling is open to the objection that the active principle may be thereby destroyed. The maternal placenta was, however, caused to form in rabbits by the use of the methods adopted by Loeb‡ when studying tissue growth in the uterus of the guinea-pig.

Pseudo-pregnant doe rabbits, obtained by coitus with a vasectomised buck, were taken at about the 7th day (the time at which the ovum normally becomes fixed in the uterine wall), and the uterus was stimulated, in some cases by snipping out pieces of the wall from each horn, or in other cases by slitting the uterus for the whole of its length and then cutting small pieces away from the edges.

The rabbits operated on in this way form decidual cells in the parts of the uterus which have been stimulated by cutting or by contact with a foreign body (figs. 2, 3, and 4). These masses of decidual cells are practically identical in appearance with those of the maternal placenta.

In each case the duration of pseudo-pregnancy dates from the day on which coitus took place with the vasectomised buck.

The results of the experiments are given below.

* Halban, 'Arch. für Gynaek.,' vol. 75 (1905).

† Basch, 'Monatsch. f. Kinderheilk.,' vol. 8 (1909).

‡ Loeb, 'Jour. Amer. Med. Assoc.,' vol. 50 (1908), and 'Arch. f. Entwick. der Organ.,' vol. 32 (1911).

(A) *Cases in which no Corpora lutea were Present in the Ovaries.**

(1) Multiparous rabbit. Small pieces of muscle, with the attached mucosa, were removed from the uterine walls at short distances apart (to represent fœtal attachments) on the 9th day after coitus. Killed on the 25th day. Mammary glands consisted mostly of ducts, with a little alveolar tissue. Openings in the uterus had closed up, and there was no decidual formation. The glands were small and the blood-vessels were not enlarged.

In the following cases (2–5) the rabbits had never been pregnant:—

(2) Small pieces of uterus removed on the 9th day after coitus. Killed on the 25th day. Ovaries very small. Uterus closed and infantile: no decidual formation. Mammary gland consisted of ducts only.

(3) Small pieces of the uterus removed on the 9th day after coitus. Killed on the 28th day. Some very old corpora lutea seen in the ovaries, which could not have been the result of the last coitus. Uterus closed; glands small; one fold of mucosa containing a few decidual cells. Mammary glands atrophic, with a little milk in the ducts.

(4) Uterine horns slit for whole length and pieces removed on the 7th day after coitus. Killed on the 30th day. Uterus closed on one side but open on the other. Mucosa showed small glands and congested capillaries, but with very little proliferation of the connective tissues. Mammary glands consisted of ducts only.

(5) Uterine horns slit and pieces removed on the 7th day. Killed on the 31st day. Uterus closed in most places. Very few glands and blood-vessels present, but one fold of the mucosa had swollen slightly. Mammary gland consisted of ducts only.

(B) *Cases in which Corpora lutea were Formed in the Ovaries.*

(6) Rabbit not previously pregnant. Uterine horns slit and pieces removed on the 8th day of pseudo-pregnancy. Killed on the 11th day. Uterus open wide, glands of the mucosa large and active, capillaries dilated, and decidual cell formation beginning in the connective tissue. Mammary glands beginning to hypertrophy, but not thickened.

(7) Multiparous rabbit. Uterine horn slit and pieces removed on the 7th day. Killed on 17th day. Uterus wide open, with large growths on the mucosa, consisting of masses of decidual cells containing hyaline material. Glands atrophic but blood-vessels dilated. Mammary glands well developed but not thickened.

* It has been noted in a former paper ('Roy. Soc. Proc.,' B, vol. 87 (1914)) that coitus is not necessarily followed by ovulation in the rabbit.

(8) Multiparous rabbit. Small pieces of uterus removed on the 8th day. Killed on the 18th day. Uterus not closed in places, but with outgrowing mucosa (fig. 2). Glands of mucosa moderately well developed, one large fold and one small one, with well developed decidual cells enclosing capillaries (fig. 3). Mammary glands well developed but not thick; milk present in the ducts.

(9) Rabbit not previously pregnant. Small pieces of the uterus removed on the 7th day. Killed on the 22nd day. Uterus open in places and decidual cells formed. Glands small, blood-vessels numerous and very dilated. Mammary glands showing signs of atrophy but not thickened.

(10) Rabbit not previously pregnant. Uterine horns slit and pieces removed on the 8th day. Killed on the 24th day. Uterus wide open (fig. 4). Glands small and capillaries dilated, and forming a plexus below the surface of the mucosa (fig. 5). Decidual cells well developed. Mammary glands well developed but not thickened.

(11) Multiparous rabbit. Uterine horns slit and pieces removed on the 7th day. Killed on the 25th day. Uterus wide open, uterine glands small, and capillaries dilated. Decidual cells formed in connective tissues, which contained small masses of hyaline material. Mammary glands well developed but not thickened.

(12) Multiparous rabbit. Small pieces of uterus removed on 9th day. Killed on the 25th day. Uterus closed, glands moderately well developed, and capillaries dilated. A few decidual cells were formed at the ends of the folds of the mucosa. Mammary glands not thickened, but contained milk, which could be expressed from the nipples.

13) Multiparous rabbit. Small pieces of the uterus removed on the 10th day. Killed on the 31st day. Uterus almost closed. Glands of the mucosa small, connective tissue dense, with slight congestion of the blood-vessels. Mammary gland in the atrophic stage.

In those cases in which, after a sterile copulation, no corpora lutea were found there was very little or no formation of decidual cells, thus confirming the opinion expressed in a former paper* that the raised nutrition of the uterus brought about by the formation of the corpus luteum is necessary for the fixation of the ova. Where corpora lutea were developed, the connective tissue cells hypertrophied and formed decidual cells, these often enclosing a large amount of hyaline material. In several cases the blood-vessels were dilated and formed a plexus supplying the surface of the mucosa (fig. 4).

* Hammond and Marshall, 'Roy. Soc. Proc.,' B, vol. 87 (1914).

Contrary to the findings of Ancel and Bouin,* we have never discovered any traces of the myometrial gland in the uteri of rabbits which have been operated on as in this series.

The effect of the formation of the decidual cells on the mammary gland is negative, no growth or glandular development taking place beyond what occurs in normal pseudo-pregnancy (see Table II).

These experiments, which were confirmed by the subsequent investigations in which the fœtuses were removed (see below), show that the maternal placenta is not the source of the stimulus which causes the glandular phase of the mammary gland.

The Fœtus.

Lane-Claypon and Starling† thought that the growth of the mammary gland in rabbits was probably due to the presence of the fœtus, and other investigators have confirmed their results. Although the actual results of their experiments have been rendered doubtful through the discovery of the action of the corpus luteum, it has not been shown that the fœtus is without effect on the mammary gland during the second half of pregnancy.

Experiments were therefore undertaken to determine the effect of removal of the fœtuses from pregnant rabbits, leaving the rest of the generative tract —ovaries, uterus, and placentæ—intact.

Weymeersch‡ has shown that the placenta persists in the uterus for some time after the embryo has been removed. Also observation of the placentæ of fœtuses which had atrophied at an early stage during development showed§ that once started the placentæ may persist during the whole course of gestation independently of the attachment of the living fœtus.

The fœtuses have been removed from pregnant rabbits between the 13th and 15th days. An incision was made through the uterus and fœtal membranes, and the fœtus withdrawn by cutting through the umbilical cord. In each case the removal resulted in the arrest of the growth of the mammary gland, and was followed shortly afterwards by the secretion of milk.

The details of these experiments are given below :—

Rabbit 1.—Fœtuses (3 and 4) removed on the 13th day after coitus. Milk expressed from nipples on the 20th day. Killed on the 25th day. Placenta still attached to the uterine wall, but showing signs of atrophy. Mammary gland not thickened.

* Ancel and Bouin, 'Compt. rend. Soc. Biol.,' vol. 72 (1912).

† Lane-Claypon and Starling, 'Roy. Soc. Proc.,' B, vol. 87 (1906).

‡ Weymeersch, 'Ann. Soc. Roy. de Science med. nat. Bruxelles,' vol. 70 (1912).

§ Hammond, 'Journ. Agric. Sci.,' vol. 6 (1914).

Rabbit 2.—Fœtuses (3 and 5) removed on the 13th day after coitus. Milk expressed on the 21st day. Killed on the 27th day. Placentæ attached to the uterine wall, three well developed, but the remainder showing signs of atrophy. Mammary glands not thickened.

Rabbit 3.—Fœtuses (4 and 5) removed on the 13th day after coitus. No milk on the 15th day. Fur pulled out and nest made on the 31st day. Killed on 32nd day, when milk was squeezed from nipples. Seven placentæ found loose in body cavity. Mammary glands not thickened.

Rabbit 4.—Fœtuses (3 and 5) removed on the 15th day after coitus. Making nest on the 20th day. Killed on the 22nd day, when milky fluid was expressed from nipples. Seven placentæ found loose in body cavity. Mammary glands not thickened.

Rabbit 5.—Fœtuses (3 and 5) removed on the 15th day after coitus. Making nest on the 20th day. Colostrum expressed on the 23rd day. Killed on the 30th day. Three atrophic placentæ attached to uterus, and four placentæ found loose in the body cavity. Mammary glands not thickened.

Rabbit 6.—Fœtuses (5 and 5) removed on the 15th day after coitus. Milk expressed on the 19th day. Killed on the 28th day. Three atrophic placentæ attached to the uterus and six free in the body cavity. Mammary glands not thickened.

It is concluded from these experiments that the fœtus is a necessary factor in causing the secondary growth changes in the mammary gland. In these cases the placentæ were retained and, in some of the animals, in a fresh condition. The results confirm those of Biedl and Koenigstein,* who found that implantation of placentæ into non-pregnant rabbits was without effect on mammary secretion, but that implantation of the fœtus resulted in the growth of the gland and the secretion of milk.

There are several facts, however, which indicate that the action of the fœtus on the mammary gland is not a direct one. Halban† has pointed out that the origin of the stimulus which causes milk secretion must be situated outside the fœtus, since the act of parturition sometimes results not only in tho secretion of milk by the mother but also by the fœtus—the so-called "witch's milk."

Moreover, many cases are known in which a woman has produced a healthy, vigorous child, and yet the mammary glands secreted very little milk. Also, as O'Donoghue‡ has pointed out, milk is secreted in the rabbit several days

* Biedl and Koenigstein, 'Zeits. f. Exp. Path. u. Therap.,' vol. 8 (1911).

† Halban, 'Arch. f. Gynaek.,' vol. 72 (1905).

‡ O'Donoghue, 'Quart. Journ. Micro. Science,' vol. 57 (1911).

before parturition, but does not occur in man and Dasyurus till several hours after it, so that the secretion of milk does not coincide with the expulsion of the fœtus. We have observed a case of a goat in which milk was secreted in large quantities (800 c.c. per day) for three weeks before the kids were born. This goat was served on September 28 and aborted on November 6; it was served again on November 20 and came into milk on April 1, the kids being born on the 21st of that month.

A further interesting fact which seems to be correlated with the diminished activity of the corpus luteum is the instinct of nest making. Often at the end of pseudo-pregnancy, at the time the animal is also "on heat," or a short time after the removal of the ovaries or fœtuses, fur is plucked out and a nest is made in preparation for the expected young.

The Corpus Luteum.

The conclusion arrived at from the previous experiments was that the growth changes of the mammary gland in the second half of pregnancy are dependent on the fœtus, but probably not directly. Since we know that the presence of the fœtus (in those animals which ovulate spontaneously) causes the corpus luteum spurium to develop into the corpus luteum of pregnancy, we should expect much the same thing to happen in the rabbit. If it does so, we might expect that the further development of the mammary gland is brought about as a result of the further growth of the corpus luteum, for it seems more likely that the mammary gland should be controlled from one point than from two, as Ancel and Bouin suggest.

The supposition that the corpus luteum of the Eutheria remains in an active state during the second half of pregnancy is not in accordance with the commonly accepted views, but O'Donoghue* has shown that the whole of the process of growth of the mammary gland and of milk secretion in the marsupial cat (*Dasyurus*) is under the influence of the corpus luteum.

He states that, in many cases after coitus where pregnancy did not supervene, the growth and activity of the mammary glands reached a stage of development indistinguishable from that which occurs in mammals a few days after parturition.

Sandes† also states that he found that the formation of the corpus luteum in Dasyurus was rapid, and that it persisted during the greater part of the time that the animal was lactating, and only disappeared when the young animals became independent.

* O'Donoghue, 'Quart. Journ. Mic. Sci.,' vol. 62 (1911).

† Sandes, 'Proc. Linn. Soc. New South Wales,' vol. 28 (1903).

Watson* has also shown that the corpora lutea of the rat during the lactation period are very large and bigger than corpora lutea spuria. He was inclined to believe that they were corpora lutea of pregnancy, which had not degenerated in the latter part of gestation.

In order to test this supposition, the corpora lutea of a series of rabbits in various conditions as regards sexual activity (pregnant, pseudo-pregnant, with decidual tissue produced, and with fœtuses removed) were measured as a test of their state of development. The method has been to cut free-hand sections of the corpora lutea in the ovaries, which in almost every case had been preserved in formalin. Three or four sections showing the greatest diameter from each corpus luteum were picked out. Their diameter was accurately measured under the low power of the microscope by means of a scale attached to the mechanical stage. In every case the largest diameter was measured. As a rule three or four corpora lutea from the ovaries of each rabbit have been measured in this way, and the results arranged so that each figure given in the Table (I) below represents the average of about sixteen measurements.

The figures given are no doubt influenced to some extent by the size and age of the rabbit, but, nevertheless, they show quite clearly that the corpus luteum of pregnancy undergoes a further development than that of the pseudo-pregnant condition.

The latter portion of the Table shows that this further development of the corpus luteum is not correlated either with the growth of the maternal placenta (as shown by the animals in which this condition was experimentally produced), or with the formation of the fœtal placenta or membranes (as shown by the removal experiments).

In order to correlate with these observations the effect of the same treatment on the mammary gland, a similar Table has been made out for this gland. The pseudo-pregnant growth of the gland is mostly in the lateral direction, so that in stained and cleared specimens one can easily see the changes occurring; but in the second stage of development the growth is rather in thickness, so that other means have to be taken to demonstrate its growth during this period.

The mammary glands have been dissected out, attached to their usual muscular band, and fixed in alcohol. The four centre glands, with the muscle beneath them, have then been cut out and weighed. This is not such a satisfactory mode of measurement as that used for the corpus luteum, for it is more dependent on the size of the animal and the amount of muscle taken with the gland. It is sufficiently accurate, however, to show that the degree

* Watson, 'Proc. Phys. Soc.,' 'Journ. of Physiol.,' vol. 34 (1906).

Table I.—Size of Corpora Lutea.

State of rabbit.	Days after coitus.															
	3–4.	5–6.	7–8.	9–10.	11–12.	13–14.	15–16.	17–18.	19–20.	21–22.	23–24.	25–26.	27–28.	29–30.	31–32.	33–34.
Pregnant	7·5	10·0	—	—	13·2	15·0	12·8	13·0 13·9 14·1 12·3 14·3 12·4	—	—	14·7 14·7	13·5 12·1 13·1 13·7	14·3 14·1 14·3 11·4 12·5	13·0 15·0 13·7 13·8	—	—
Average	7·5	10·0	—	—	13·2	15·0	12·8	13·3	—	—	14·7	13·1	13·3	13·9	—	—
Pseudo-pregnant ...	—	—	—	9·2	—	13·2	11·6	10·8 11·6 8·0	—	—	9·2 11·2	—	6·7 8·5	—	—	—
Average	—	—	—	9·2	—	13·2	11·6	10·1	—	—	10·2	—	7·6	—	—	—
With experimentally produced decidual cells	—	—	—	—	10·5	—	—	12·2 11·6	—	—	—	8·8 7·5	6·7	—	7·2	—
Average	—	—	—	—	10·5	—	—	11·9	—	—	—	8·1	6·7	—	7·2	—
With fœtus removed	—	—	—	—	—	—	—	—	—	9·2	—	8·3	8·2 8·1	7·6	—	8·7
Average	—	—	—	—	—	—	—	—	—	9·2	—	8·3	8·2	7·6	—	8·7

Table II.—Weight of Mammary Gland (Grm.)

State of rabbit.	Days after coitus.															
	3–4.	5–6.	7–8.	9–10.	11–12.	13–14.	15–16.	17–18.	19–20.	21–22.	23–24.	25–26.	27–28.	29–30.	31–32.	33–34.
Pregnant............	13·5	11·0	—	12·5	—	—	—	18·6 18·6 11·5 14·0	—	—	16·7 20·5	32·3 30·2	38·4 29·7 45·0 37·3 27·7 39·8	49·7 31·4 41·6 45·3	—	—
Average..........	13·5	11·0	—	12·5	—	—	—	15·7	—	—	18·6	31·2	36·3	42·0	—	—
Pseudo-pregnant ...	—	—	—	—	—	—	—	11·0 16·0	—	—	—	16·0	12·6	—	—	—
Average..........	—	—	—	—	—	—	—	13·5	—	—	—	16·0	12·6	—	—	—
With experimentally produced decidual cells	—	—	—	—	8·5	—	—	21·0 14·0	—	8·5	11·5	15·5 18·5	16·5	10·5	10·5	—
Average..........	—	—	—	—	8·5	—	—	17·5	—	8·5	11·5	17·0	16·5	10·5	10·5	—
With fœtus removed	—	—	—	—	—	—	—	—	—	24·0	—	19·5	14·5 18·0	14·5	—	12·0
Average..........	—	—	—	—	—	—	—	—	—	24·0	—	19·5	16·2	14·5	—	12·0

Fig. 1

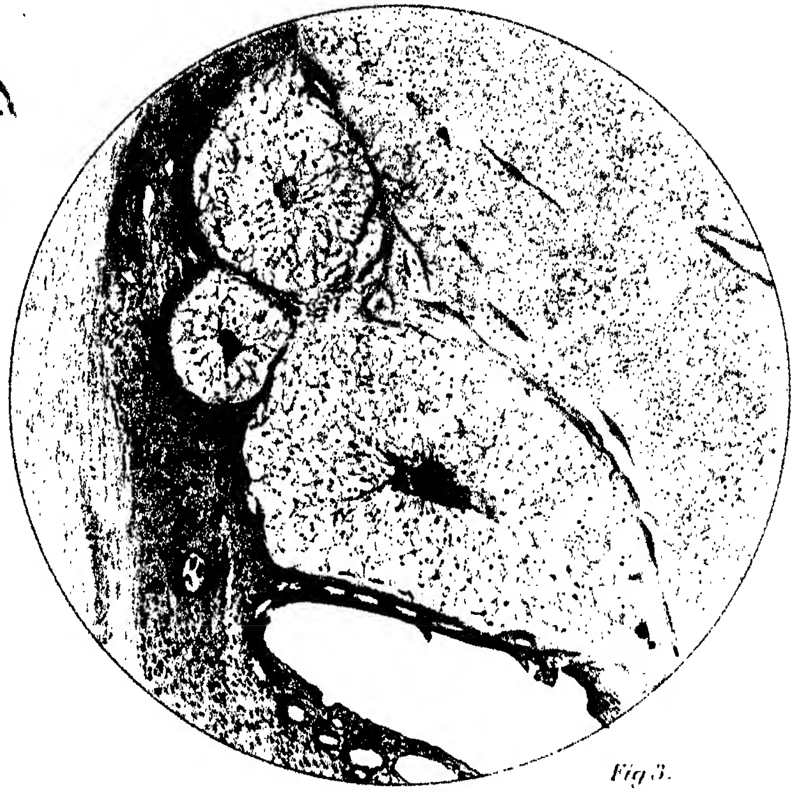

Fig. 3.

Fig. 2.

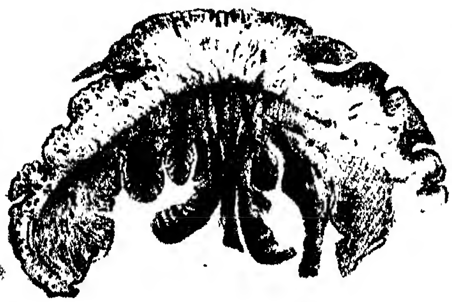

Fig. 4

Fig. 5.

of development of the gland in the pregnant and the pseudo-pregnant condition is quite distinct.

Table II shows the results obtained. Comparing this with Table I it is seen that there is a definite correlation between the development of the corpus luteum and the increase in weight of the mammary gland.

Summary and Conclusions.

1. Experimental results show that the development of the mammary gland of the rabbit during the second half of pregnancy is under the same influence as that which controls the development during the first half, namely, the corpus luteum.

2. Contrary to the generally accepted opinion, this gland (the corpus luteum) is active during the second half of pregnancy.

3. The further development of the corpus luteum, which takes place during the latter part of pregnancy, is due to the influence of the fœtus.

4. The experiments do not uphold the view of Ancel and Bouin that the glandular phase of the mammary gland is due to something entirely different from that which causes the growth-changes, but confirm the views expressed in an earlier paper by Hammond and Marshall, in which it was shown that milk secretion in pseudo-pregnancy takes place in correlation with the involution of the corpus luteum.

Apparently the secretion of milk results whenever the influence causing the glandular growth (the corpus luteum) is removed or lessened in amount, provided that the initial development has gone far enough.

The experiments described above were performed in 1913–14 at the School of Agriculture and Field Laboratories, Milton Road, Cambridge. The expenses were, for the most part, defrayed by a research grant from the Board of Agriculture and Fisheries out of funds placed at their disposal by the Development Commissioners.

I am greatly indebted to Dr. F. H. A. Marshall for the help he has given me in preparing this paper.

DESCRIPTION OF PLATE.

[The figures were drawn by the late Edwin Wilson, of Cambridge.]

Fig. 1.—Giant cells referred to on p. 536. [This section also passed through the myometrial gland, and it was intended that the cells of this gland should be shown on the figure, but, unfortunately, owing to inability to supervise the drawing, as a result of the outbreak of war, and the subsequent death of the artist, the figure was not completed.]

Fig 2.—Experimentally produced Placenta—Section of Uterus, showing Outgrowing Lobe of Mucosa, where small piece has been removed by operation. (Low power.)

Fig. 3.—Experimentally produced Placenta—Connective Tissue forming Decidual Cells which enclose Blood Capillaries. (Same section as fig. 2, more highly magnified.)

Fig. 4.—Experimentally produced Placenta—Part of Section of Uterus, which has been slit open, showing Growth of Placental Tissue in upper portion of section. (Low power.)

Fig. 5.—Experimentally produced Placenta (same section as fig. 4, more highly magnified)—Plexus of Blood-vessels below the Surface of the Mucosa.

On the Post-Œstrous Changes occurring in the Generative Organs and Mammary Glands of the Non-Pregnant Dog.

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[Plates 24–26.]

The œstrous cycle in animals which come "on heat" at relatively infrequent intervals (*i.e.* monœstrous animals) has been divided by Heape into the following four periods:—

(1) Anœstrum (period of rest).

(2) Pro-œstrum (periods of growth and destruction).

(3) Œstrus (period of desire).

(4) Metœstrum (period of recuperation, occurring only in the absence of pregnancy).

This scheme of classification was adopted by Marshall and Jolly in describing the changes occurring in the generative organs of the Bitch. It has been shown that ovulation normally takes place during œstrus at or near the termination of the sanguineous discharge which characterises the pro-œstrum. This observation has since been confirmed by Keller. Ovulation occurs spontaneously in bitches, the additional stimulus of coitus being unnecessary.

In the Bitch, as in all mammals, ovulation is followed by the formation of the corpus luteum. This structure is usually described as degenerating within a comparatively short period if pregnancy does not supervene after coitus (corpus luteum spurium), but remaining persistent throughout the whole or greater part of gestation when this condition follows (corpus luteum verum). According to Ancel and Bouin, however, in the Rabbit, in which ovulation takes place normally as a result of coitus (Heape), there apparently is only one kind of corpus luteum (the corpus luteum verum), which structure can be induced to form experimentally in the absence of pregnancy by employing vasectomised males. Ancel and Bouin have shown that, under such circumstances, (condition of pseudo-pregnancy),* the uterus undergoes glandular development and vascularisation, while the tissue of the mammary glands proliferates rapidly, the hypertrophy continuing until about the 15th day, when the corpora lutea begin to degenerate. O'Donoghue and others have confirmed these results for the mammary gland. The development of the mammary tissue in the later part of (true) pregnancy is ascribed by Ancel and Bouin to the influence of the myometrial gland, but Hammond has shown that it is far more likely to be due to the continued influence of the corpora lutea, depending upon the presence of the fœtus. According to this observer, the corpora lutea do not degenerate in the later part of pregnancy, and, consequently, the corpora lutea of pseudo-pregnancy in the rabbit are comparable to the corpora lutea spuria of most other mammals, although it would seem probable that (when produced) they persist for a longer time, and exert a greater influence than in those polyœstrous animals which ovulate spontaneously.

In the Marsupial Cat (*Dasyurus*) there is only one kind of discharged follicle, the corpus luteum of pregnancy or pseudo-pregnancy, and the changes which occur in the mammary glands as a result of luteal influence are identical, irrespectively of whether gestation supervenes or not (Hill and O'Donoghue).

In polyœstrous animals the corpus luteum spurium usually degenerates after a short period, so as to make way for the maturation of new follicles and the process of ovulation at the frequently recurring œstrous periods. Otherwise, the ripening follicles degenerate under the influence of the corpus luteum. Sandes has described follicular atrophy in widening circles around the fully developed corpus luteum of *Dasyurus*. The more rapid degeneration of the corpus luteum spurium has probably taken place in

* The term "pseudo-pregnancy," to describe the experimentally produced condition, was first used by Hammond and Marshall, who confirmed Ancel and Bouin's descriptions of the uterine changes occurring under luteal influence in the Rabbit.

association with the acquirement of the polyœstrous habit, since it would be detrimental to fecundity if these structures persisted for as long a time as corpora lutea of pregnancy. It is equally evident, however, that in monœstrous animals, such as the Dog, the persistence of the corpus luteum spurium over a considerable period would not exercise the same prejudicial effects as in polyœstrous animals.

It became of interest therefore to ascertain the duration of the corpus luteum spurium in the Dog, and to discover whether, if this organ persists for a longer period than in polyœstrous animals, the continuance is associated with uterine and mammary development comparable to what occurs in the pseudo-pregnant Rabbit and in the Marsupial Cat. Accordingly we took a number of bitches (mostly virgins) which were not permitted to become pregnant, and killed them at varying intervals after "heat," so as to acquire a series of stages showing different degrees of development in the generative organs and mammary glands.

The animals were kept under observation for prolonged periods, and there could be no doubt about the occurrence of the "heat" periods from which the stages of development were dated, for in each case there was a very definite sanguineous discharge of normal duration. Certain of the bitches, however, had at previous times shown very slight indications of heat, as manifested by congestion of the vulva accompanied by a mucous discharge. In view of these observations, little or no importance should be attached to the transitory appearances suggestive of heat which have been recorded as occurring after the injection of ovarian extracts* (Marshall and Jolly).

The following are the records of the cases dealt with in the present investigation:—

(1) A half-bred Whippet (a virgin) began to show pro-œstrous bleeding from the vaginal opening on May 17. It stopped bleeding on May 31. On June 2 it was killed, and the generative organs and portions of tissue from the mammary region were preserved. The right ovary contained three discharged follicles, and the left ovary two. Sections through the discharged follicles showed that they were of very recent origin, ovulation having taken place probably within the previous two days. The corpora lutea were not organised, the ingrowth of connective tissue from the follicular wall was relatively slight, the cavity was only very partially and irregularly filled in,

* In a former investigation by Carmichael and Marshall, experiments with commercial ovarian extracts were entirely negative. The transplantation experiments by Marshall and Jolly, upon which the views put forward were mainly based, belong to a different category, for in these cases heat of normal duration and character occurred. Transient signs of heat were never observed after ovariotomy had been performed.

and the point of rupture was clearly visible. In the cavities there were many signs of recent hæmorrhage, for red corpuscles and blood pigment were abundant. The cells destined to contain lutein had already undergone some hypertrophy, while connective-tissue cells and small blood-vessels had grown inwards from the surrounding tissue.

Sections through the uterus showed extravasated blood in considerable quantity, lying, for the most part, a little below the surface epithelium (*cf.* Marshall and Jolly, and Keller). There was no clear evidence of destruction of epithelium having occurred, but the duration and extent of external bleeding on the preceding days is proof of a considerable discharge of blood having taken place from the mucosa into the lumen of the uterus. The superficial epithelium was cubical; that of the crypts opening directly into the lumen of the uterus was more columnar and showed evidence of mitosis. The glands situated in the deeper portion of the stroma were also lined by a more or less columnar epithelium. They were slightly more numerous than in the pro-œstrous stage (described by Marshall and Jolly, and Keller), and their lumina were very widely open. The stroma was denser than during the pro-œstrum.

The mammary gland tissue was limited almost entirely to a few ducts in the neighbourhood of the nipple, and there was no evidence of proliferation taking place in such mammary tissue as was present.

(2) A rough-haired Terrier (a virgin) began to show pro-œstrous bleeding from the vaginal opening on May 21. Bleeding continued until June 2, when it seemed to have stopped. Slight bleeding was, however, resumed next day and continued until June 6, when it finally ceased. The bitch was killed on June 10, when her organs were preserved. Each ovary contained four organised corpora lutea, which may have been about a week old. The prolonged continuance of slight bleeding from the vulva seemed to suggest that in this case œstrus had commenced and ovulation taken place before the discharge had quite ceased. Otherwise the pro-œstrum had been of abnormal duration. The degree of development of the corpora lutea supported the first suggestion, for, excepting for the fact that the cavities were not entirely filled in, these organs were fully formed. They occupied a large part of the ovary, and were well vascularised.

The sections through the uterus showed a slight increase in the number of glands in the deeper portion of the stroma, as compared with the stage described above. The epithelium lining the crypts and glands was throughout columnar rather than cubical, the nuclei being situated in the basal portions of the cells. The lumina of the glands were not so widely open as in the preceding stage. The connective tissue was fairly dense, but somewhat

less so in the central and deeper portions of the stroma. There was no longer any sign of hæmorrhage, and no pigment was discernible.

The mammary tissue showed very definite growth, being spread over a considerable area, and not merely in the immediate neighbourhood of the nipple. The lobes were constituted by numerous mammary acini separated by connective tissue. There was every indication of rapid development proceeding.

(3) A multiparous brindled Terrier underwent pro-œstrous bleeding from May 15 until May 23. It was killed on June 2, or ten days later. The right ovary contained three or four corpora lutea, and the left ovary three corpora lutea. Sections of one ovary showed that the corpora lutea were fully developed and highly vascularised.

The uterus was also highly vascular, the blood being contained within capillaries situated in the middle portion of the stroma and between the glands. The latter were very numerous, especially in the peripheral region of the stroma, where there was little intervening connective tissue. They were lined by a columnar epithelium (fig. 2).

The mammary tissue, as was to be expected, was well developed, but the animal being a multiparous one was unsuitable for purposes of comparison with virgins in which the gland tissue showed different degrees of growth.

(4) A Terrier bitch (a virgin) experienced pro-œstrous bleeding from April 29 until May 7. Very slight bleeding continued until May 12. The bitch was killed on June 2. The right ovary contained six corpora lutea and the left ovary four. Each ovary was of relatively large size. Sections showed that the ovaries consisted mainly of corpora lutea, which appeared to have reached their full development. The luteal cells were much hypertrophied.

The uterine mucous membrane contained vast numbers of glands, but these were for the most part smaller and had a lesser diameter than in the stages described above. The peripheral part of the mucosa was especially packed with glands, but trabeculæ of stroma tissue divided the glandular elements in the more central part of the mucous membrane. The epithelium surrounding the glands was still markedly columnar. Their lumina almost invariably contained a colloidal substance which stained red with eosin. There was a very considerable capillary development throughout the connective tissue.

The mammary tissue at this stage had developed appreciably. There was a well-marked growth (in some places quite dense) for about ¾ inch around each nipple. Sections showed secretory acini in a further stage of development, and cellular proliferation was proceeding. The intermammary tissue was highly vascular, and there was a large quantity of fat present (fig. 6).

(5) A Fox Terrier (a virgin) experienced pro-œstrous bleeding from February 2 to February 9 and was killed on March 11, or 31 days after bleeding ceased. Both ovaries appeared to be composed very largely of luteal tissue. It was subsequently found that the right ovary contained two corpora lutea and the left ovary three. Sections showed that the corpora lutea were still well vascularised but beginning to undergo very slight retrogression. The luteal cells were much hypertrophied but vacuoles were beginning to appear in some parts of the tissue.

The uterus also showed slight evidence of retrogressive changes having set in. The crypts and glands were smaller. The epithelium lining them was less often columnar and in places was almost or quite cubical. The gland lumina frequently contained a colloidal substance, and in the case of some glands there were appearances which suggested a recent desquamation of epithelial cells. Vessels were abundant in the interglandular tissue, but the capillary development was less noticeable than in the preceding stage. The muscular walls, however, showed little or no evidence of having undergone any change.

The development of mammary tissue was more prominent than in the preceding stage, and active proliferation was apparently still proceeding. Large numbers of acini had been formed (figs. 5 and 7). No fat was present in the intermammary tissue, but this was probably mainly a result of the animal's condition. The tissue was not markedly vascular, but vessels could be distinctly seen.

(6) A Fox Terrier (multiparous) experienced pro-œstrous bleeding from August 4 till August 14 and was killed on September 21, or 38 days later. Superficial examination showed that the right ovary contained two corpora lutea and the left ovary three. The ovaries unfortunately were not preserved, so that it is not possible to describe the histology of the luteal tissue.

Sections through the uterus presented several differences from those of the preceding stage. The interglandular connective tissue constituted the greater part of the area of the stroma, the glands being both fewer and smaller in diameter. Small capillaries containing red corpuscles were numerous in the stroma. The glands throughout were lined by cubical epithelium, the nuclei being placed centrally and occupying about two-thirds of each cell as seen in section. The gland lumina contained colloidal substance, and here and there were portions of what were probably remains of epithelial cells (fig. 3).

The bitch being multiparous, the mammary tissues were well developed and consequently unsuitable for comparison with the stages described above. No secretion or fluid could be expressed from the nipples.

(7) A long-haired Terrier (multiparous) experienced pro-œstrous bleeding

from May 29 to June 8, when bleeding appeared to have stopped. A very slight sanguineous discharge was, however, observed on June 10, 11, 12, and 13. The bitch was killed on July 26, 48 days after June 8, which probably marked the end of the pro-œstrum, or 43 days after June 13, when all signs of bleeding finally ceased. Fluid could be expressed from the nipples, the mammary glands being well developed.

The right ovary was seen to contain at least two corpora lutea. The left ovary contained three corpora lutea, which occupied a large part of the organ. Sections showed that the luteal cells were still large but much vacuolated, and undergoing degeneration, but the nuclei were very distinct.

The uterus presented a stage of retrogression considerably more advanced than the previous stage. The epithelium lining the glands in some parts (especially in the deeper parts of the mucosa) had undergone almost complete destruction, that which was left was cubical and never columnar. The cavities of some of the glands contained colloid and what were probably the remains of desquamated epithelial cells. The changes which had taken place had affected the blood supply, for many of the capillaries had broken down, and there were corpuscles freely extravasated in the stroma (fig. 4). The sections showed a resemblance to the late pseudo-pregnant stage described by Hill and O'Donoghue for *Dasyurus*.

Apart from the breaking-down of vessels and the presence of extravasated blood in the stroma, the uterus showed no resemblance to the pro-œstrous uterus, the condition of the glands being very different; for, whereas the glandular epithelium of the pseudo-pregnant uterus at this stage is degenerate, or else new and attenuated, that of the pro-œstrous uterus is columnar. Moreover, in the sections of the pseudo-pregnant uterus there was no evidence of blood passing into the uterine cavity, and there had been no external bleeding from the vaginal opening of this bitch before killing.

Sections through the mammary glands showed that these were in a state of activity, the alveoli containing a quantity of fluid, but we failed to identify fat by staining with Sudan III. Since the bitch was multiparous the sections were unsuitable for comparison with those of the virgin animals described above, but it is significant to note that the glands must have undergone growth in order to be in a condition of secretory activity.

(8) This was a Fox Terrier, which was apparently a virgin. It had probably been "on heat" at least once, but during the three months during which it was under observation, "heat" had not occurred. The ovaries contained numerous developing follicles but no luteal tissue. The uterus was small, the mucosa relatively thin and with few glands or vessels. The glands present were very small and lined by a cubical epithelium. The

mammary tissue was chiefly represented by ducts in the neighbourhood of the nipple.

It is necessary to assume that the uterine glands undergo considerable increase in size and number with the approach of the pro-œstrum, for at this period they are well represented and lined by a columnar epithelium (fig. 1).

The changes which occur in the œstrous cycle of the Dog were first briefly described by Retterer, whose account was afterwards amplified and to a great extent confirmed by Marshall and Jolly. The postœstrous changes, however (excepting for the first few days after "heat"), were not recorded, it being supposed that the uterus rapidly resumed the resting condition. The postœstrous glandular hyperplasia was first described by Keller, who has supplied a very careful account of the changes in the uterus from the pro-œstrum onwards until rest. This investigator obtained a series of stages until the eleventh week by removing portions of the uterine wall from living bitches at varying intervals and then allowing the cycle to continue. In this way he procured a succession of stages showing the growth and increase in the glands and the subsequent retrogressive changes, the occurrence of which is confirmed in the present paper. Keller's records, obtained in the way described, were supplemented from some further material obtained promiscuously. Keller gives an account of the changes undergone by the epithelium of the crypts and glands, and records the existence of a secretion within their lumina during the "rückbildung" or retrogressive stage. Certain of Keller's stages, however, were taken at somowhat long intervals, and this may account for his having missed altogether the extravasation stage represented in Case 7. The paper is written with especial reference to the existence of endometritis in the human uterus. The part played by the corpus luteum in relation to the changes and their physiological significance are not considered, and there is no record of the synchronous processes undergone by the mammary tissue.

The changes in the uterine glands and stroma tissue of the pseudo-pregnant dog are in a general way very similar to those of the marsupial as described by Hill and O'Donoghue. These authors point out that the processes which occur in the pseudo-pregnant marsupial are essentially identical with those which take place in the pregnant animal. A similar statement can be made about the Dog, as is shown by comparing Duval's description and figures of the uterine mucosa at different stages of pregnancy. The extent of the glandular development (the object of which is to help to provide nourishment from the secretion produced for the growing embryos

when present) is probably not so great in the pseudo-pregnant animal, but is otherwise of the same general character.

Ancel and Bouin were the first to show that the uterus undergoes active changes in response to experimentally produced corpora lutea in the pseudo-pregnant rabbit. They describe glandular hypertrophy and increased vascularisation, followed by retrogression. The occurrence of these changes was confirmed by Hammond and Marshall, who have pointed out their essential similarity with the pseudo-pregnancy processes in the uterus of the Marsupial Cat (*Dasyurus*), as recorded by Hill and O'Donoghue. Hammond and Marshall show further (what is a corollary of the last statement) that these changes in *Dasyurus* are not comparable to the pro-œstrous phenomena of the Eutheria generally, as Hill and O'Donoghue supposed them to be.* Neither Ancel and Bouin, nor Hill and O'Donoghue, knew of Keller's work on the Bitch (confirmed and extended in the present paper), and it is left to us to point out the parallelism between the postœstrous processes in the three mammals in which they have been described.

Ancel and Bouin, and other investigators, have shown that in the Rabbit the postœstrous uterine development only takes place in the presence of corpora lutea, which can be induced to form, without supervention of pregnancy, by employing vasectomised males (*i.e.* under a condition of experimentally induced pseudo-pregnancy). Moreover, the parallel series of characteristic changes both in *Dasyurus* and in the Dog are always associated with the development and subsequent retrogression of the corpora lutea.† There can be little doubt, therefore, that the corpus luteum is an essential factor in the hyperplasia of the uterine glands and other correlated changes in these animals, just as it is in the Rabbit. To prove this definitely in the case of the Dog, it would be necessary to destroy the luteal tissue while leaving the rest of the ovaries, but to perform this operation upon the ovaries lying *in situ* in the body was, in our judgment, impracticable.

The question arises as to whether there is any phase in the menstrual cycle of Man which corresponds to the final or destruction stage of the pseudo-pregnant period of the Dog. Bryce and Teacher and other authors

* In the paper referred to we inadvertently omitted to note that in the scheme of comparison drawn up by Hill and O'Donoghue to describe the cycle of changes in the Marsupial and in the Eutherian the terminology is that of Heape, who was the first to deal systematically with the phases of the œstrous cycle. Hill and O'Donoghue, however, are alone responsible for identifying the uterine degenerative changes of the pseudo-pregnant *Dasyurus* with the pro-œstrous changes of the Eutherian.

† According to Moreaux, the presence of corpora lutea in the Rabbit's ovaries is associated with an excretory phase on the part of the uterine glands.

have shown that ovulation, when it occurs in Man, probably takes place shortly after the menstrual period is over. It is usually stated (*cf.* Halliburton's 'Handbook of Physiology') that the corpus luteum spurium in Man is in a condition of retrogession at the end of one month, being smaller then than after a period of three weeks. It has been established by Heape and other investigators that menstruation in the Primates is the homologue of the pro-œstrum in the lower Mammalia. Nevertheless, it seems possible, in the light of the facts described in this paper, that the menstrual cycle is a process of greater complexity than most authorities have supposed, and that the destruction stage corresponds partly to the later portion of the pseudo-pregnant period of the Dog, when the corpus luteum no longer exerts an anabolic influence upon the reproductive organs. If this surmise is correct, it helps to reconcile the two seemingly divergent views as to the homologous stages of the cycle, namely, the view of Heape, according to whom the pro-œstrum and menstruation are identical (a view taken in previous papers by Marshall), and the theory put forward by Beard, and adopted in more recent publications by Grosser, Hitschmann and Adler, and Hill and O'Donoghue, according to whom the menstrual process represents the removal of a preparation made for a fertilised ovum which failed to arrive, or a degeneration of uterine tissue which was unable to fulfil its purpose. In this connection it is interesting to note that, according to Hitschmann and Adler, the premenstrual uterus in Man undergoes changes which are similar in character to those observed in the pregnant uterus. It seems possible, therefore, to regard menstruation in Man as representing pseudo-pregnant destruction as well as pro-œstrous degeneration, owing to the whole cycle of changes having been compressed into one month. Indeed, unless some such explanation as this be adopted, one must suppose that the processes of pseudo-pregnancy are unrepresented in the menstrual cycle, and that the corpus luteum spurium in the Primates exerts little or no influence.

Since the retrogressive changes in the uterus of *Dasyurus*, the Rabbit and the Dog in the later part of pseudo-pregnancy are correlated with the degeneration of the so-called corpus luteum spurium, it seems reasonable to suppose that the retrogression of the corpus luteum verum at the close of gestation may be one of the factors involved in those destructive changes which are associated with parturition.

It has been pointed out that the comparatively long persistence of the corpora lutea spuria and their influence upon the uterus and mammary glands in bitches are probably associated directly with the monœstrous habit. In most polyœstrous animals the corpus luteum is believed to

degenerate after a shorter period. This statement is certainly correct for some species, but the matter is one which requires extended study on comparative lines.

The similarity between the developmental progress of the mammary tissue occurring in the postœstrous non-pregnant Dog and that taking place under experimental conditions in the pseudo-pregnant Rabbit is evidence that the mammary development, like the uterine development, is dependent in both animals upon the influence of luteal tissue. It should, however, be made clear that in the cases under observation the mammary growth was limited, and that in pregnancy some further factor must come into play to complete the development. The probable factor concerned in the case of the pregnant rabbit is the subject of a communication by Hammond.

In another paper Hammond and Hawk have remarked on the tendency for the milk record of a cow to fall at about the time of œstrus, and it seems possible that such periodic variation in milk secretion may depend partly upon an anabolic influence on the part of the corpus luteum if the development of this organ in the Dog, the Rabbit, and *Dasyurus* is correlated with a building up of mammary isssue, rather than with the actual secretion of milk. Furthermore, it is known that in the Sheep, which, like the Cow, is polyœstrous, the corpus luteum spurium attains its maximum development very rapidly, being soon succeeded by degenerative changes, so that such influence as this organ may possess upon the uterus and mammary glands must be of very brief duration.

Lastly, reference may again be made to the observations recorded by Heape, Noël Paton, Blair Bell, and others of non-pregnant bitches (in some cases virgins) secreting milk several weeks after œstrus, and frequently at about the time when they would have given birth had they been pregnant. Mr. F. Reynolds, of Devizes, has informed us that he also has observed instances of the same phenomenon, and we have reason to believe that such cases among bitches are far commoner than is ordinarily known. They are to be explained as following upon an increase in certain individuals in the activity of the corpora lutea.

Steinach and Athias have recorded mammary growth as a result of transplanting ovaries into previously castrated male guinea-pigs, but to what precise ovarian elements the influence is to be attributed has not been made clear. Steinach states that the mammary glands reached a degree of development comparable to that of pregnant females, and sufficient to admit of the manufacture of milk and suckling of young, fat globules being found in the secreted fluid.

Conclusions.

The uterus and mammary glands of the non-pregnant Bitch undergo pronounced postœstrous development under the influence of the corpora lutea, there being a definite pseudo-pregnant period.

Retrogressive changes do not set in with any of these organs until about 30 days after ovulation, and in the case of the mammary glands a somewhat later period.

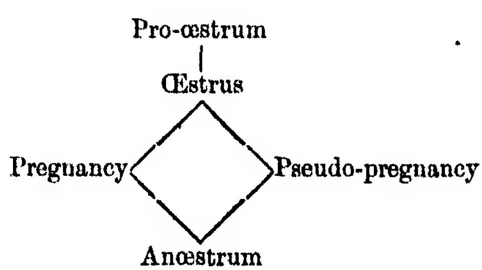
The developmental changes are of a similar kind to those taking place during pregnancy, but do not reach the same degree of development.

The entire series of changes are physiologically homologous with the changes shown by the uterus and mammary glands of the pseudo-pregnant Rabbit and Marsupial Cat.

The relatively long persistence of the corpora lutea in the Bitch is probably correlated with the monœstrous habit.

This persistence, which is possibly greater in some individuals than in others, elucidates the not uncommon phenomenon of bitches which had not been impregnated secreting milk at or near the end of the pseudo-pregnant period.

The changes which occur in the generative organs and mammary glands after œstrus are now brought into relation with the rest of the œstrous cycle, and the complete cycle in the Bitch may be summarised in the following scheme:—



The terms pro-œstrum, œstrus, and anœstrum are those originally proposed by Heape, and represent the periods so described by him. The first part of the anœstrum is generally occupied by the nursing or lactation period, but in the case of animals which have experienced pseudo-pregnant conditions the lactation period is usually only very imperfectly represented. The metœstrous period must now be regarded as unrepresented in the Bitch. It exists in animals which do not experience pseudo-pregnancy (*e.g.* in those rabbits in which corpora lutea are not formed after œstrus).

The work was carried out at the School of Agriculture and Field Laboratories, Cambridge. The expenses were defrayed largely by a grant

from the Board of Agriculture and Fisheries out of funds placed at their disposal by the Development Commissioners.

[*Added June* 6, 1917.—Leo Loeb states that there is a definite cycle in the non-pregnant Guinea-pig for the mammary gland, and that it corresponds with the ovarian and uterine cycles. The gland tissue proliferates when a new ovulation is imminent or in the presence of corpora lutea. Extirpation of the corpora lutea was followed by an inhibitory effect.

Pearl has suggested that the corpus luteum is one of the chief agents in maintaining the female secondary characters, since a cow began to assume the characters of the male upon ceasing to ovulate, although the germinal and interstitial tissue of the ovaries remained normal. He states also that the corpus luteum has an inhibitory effect upon ovulation, this conclusion being based partly upon the effects of injecting luteal extract into fowls.]

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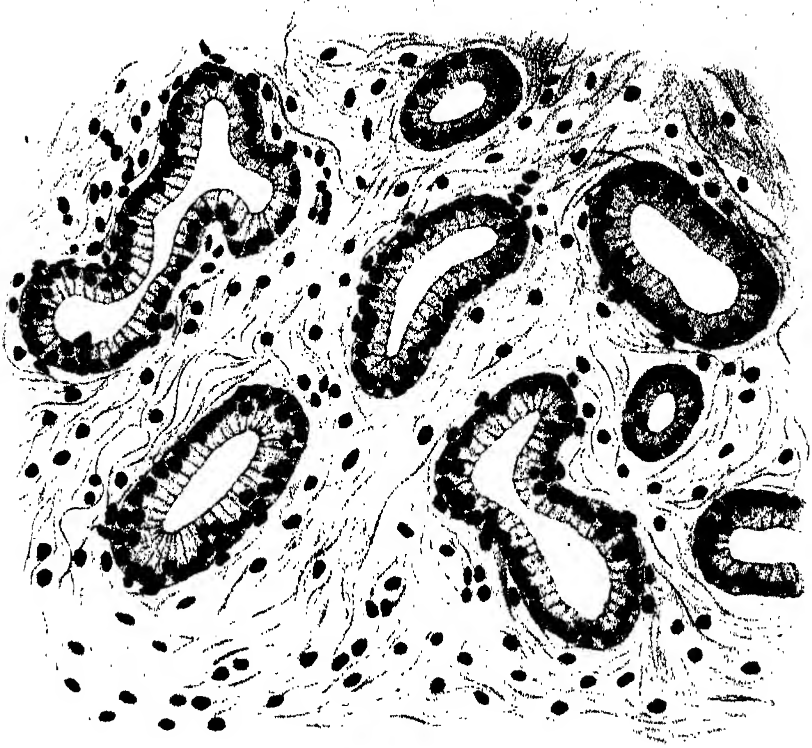

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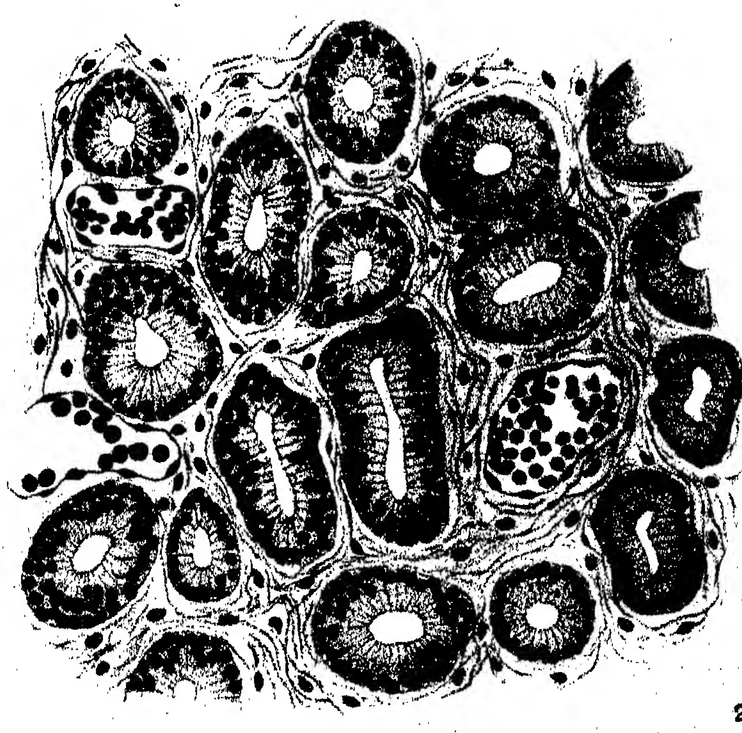

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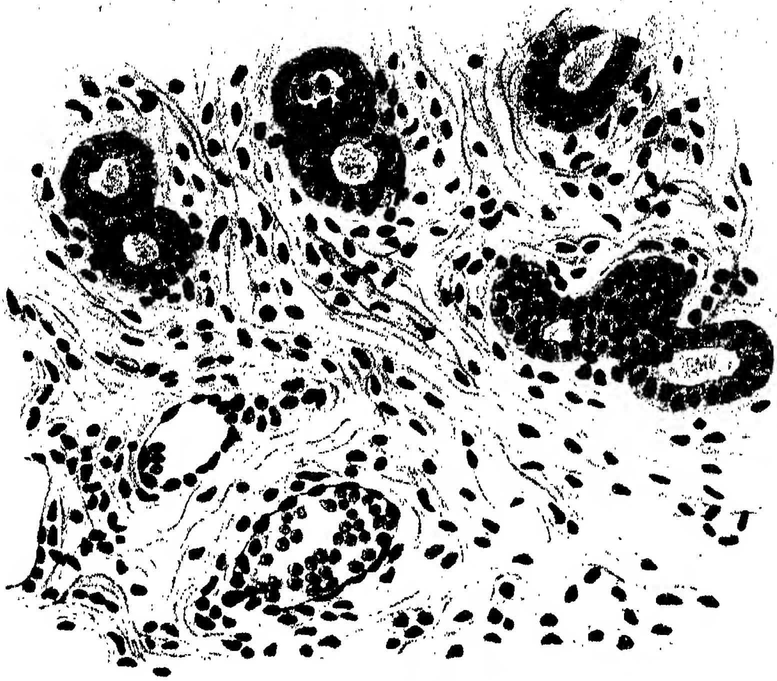

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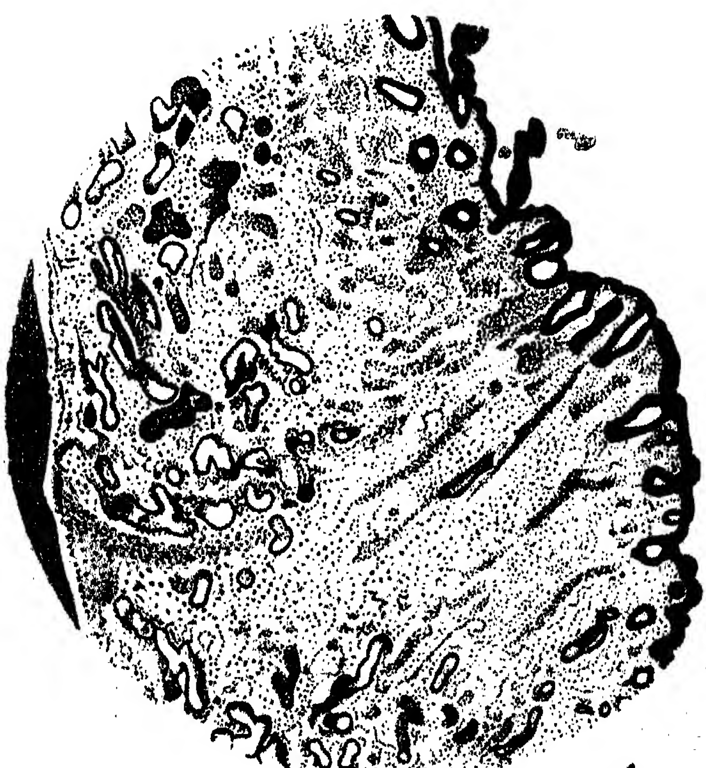

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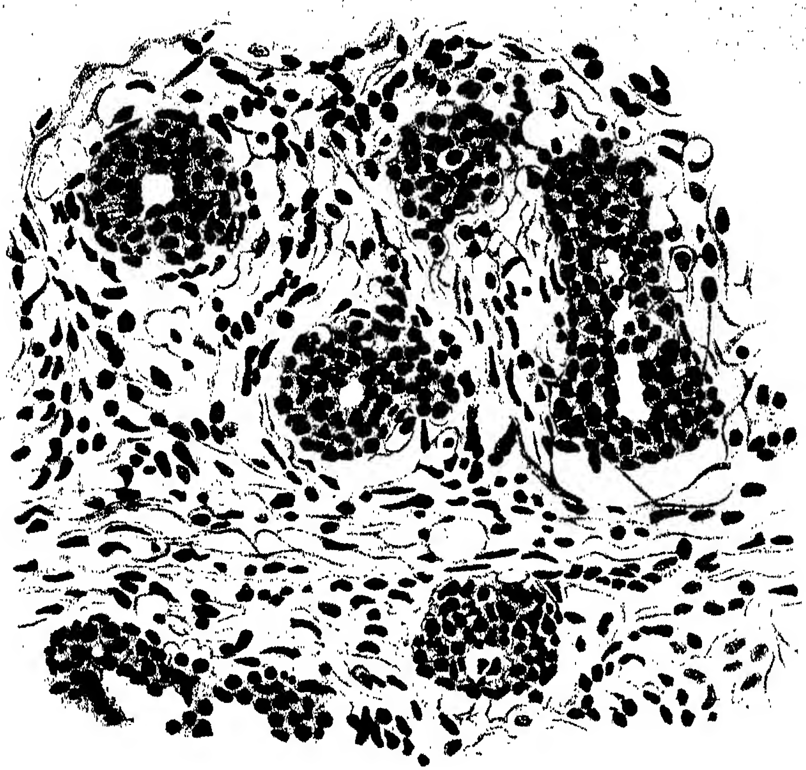

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DESCRIPTION OF FIGURES.

(The figures were drawn by Mr. Parker, of the Cambridge University Press.)

Plate 1.

Fig. 1.—Section through pro-œstrous uterine stroma (high power). The figure represents a portion of the deeper part of the stroma where extravasated blood corpuscles were *not* present. In the more superficial part underlying the surface epithelium such corpuscles were abundant. The uterine glands show a high columnar epithelium.

Fig. 2.—Section through uterine stroma of Bitch No. 3 (high power). The glands are very well developed and closely packed, there being comparatively little intervening connective tissue. The glandular epithelium is columnar. Blood-vessels are fairly numerous.

Plate 2.

Fig. 3.—Section through uterine stroma of Bitch No. 6 (high power). The glands have undergone a change. The epithelium is now usually cubical. The lumina contain colloid and sometimes what appear to be desquamated epithelial cells.

Fig. 4.—Section through uterine mucosa of Bitch No. 7 (low power). The glands are for the most part in a state of degeneration. Their lumina contain colloid, together with the remains of epithelial cells. Extravasated blood is present in the stroma.

Plate 3.

Fig. 5.—Section through mammary tissue of Bitch No. 5 (high power), showing alveoli.

Fig. 6.—Microphotograph (low power) of section through mammary tissue of Bitch No. 4, showing ducts, alveoli, and a quantity of adipose tissue.

Fig. 7.—Microphotograph (low power) of section through mammary tissue of Bitch No. 5, showing numerous alveoli undergoing proliferation. Some ducts are also shown.

Croonian Lecture.—*Upon the Motion of the Mammalian Heart.*

By Thomas Lewis.

(Received May 1, 1917.)

In these days when Europe is ablaze from end to end and our armies are fighting to regain that freedom and peace which a few years back enabled us undisturbed to pursue our search for knowledge, I wish that this lecture could deal with some problem which affects the health of our troops. But though engaged for some while with my fellow workers upon problems of this kind, I am unable to deliver the lecture in this form. Were I to attempt it with the material at my disposal, it would not be compatible with the traditions of this lectureship to which you have done me the honour of appointing me. As an alternative permit me to review a chapter of physiology recently brought to completion and one which we may fairly claim to have been compiled in chief part by workers in this country.

Three hundred years have passed since William Harvey, our fellow countryman, preached the doctrine by which his name has been immortalised. In his book 'De Motu Cordis,' that famous model of unclouded thought and of scientific reasoning, he wrote of the mammalian heart in these words:—

"First of all, the auricle contracts, and in the course of its contraction throws the blood into the ventricle, which being filled, the heart raises itself straightway, makes all its fibres tense, contracts the ventricles, and performs a beat, by which beat it immediately sends the blood supplied to it by the auricle into the arteries; these two motions, one of the ventricles, another of the auricles, take place consecutively, but in such a manner that there is a kind of harmony or rhythm preserved between them."

It is that sequence of movement, it is that harmony or rhythm of which Harvey wrote, that forms the subject of this lecture.

Our knowledge of the heart's movements progressed but slowly from the time of Harvey's discoveries. In the days of Albrecht Haller, the Swiss, the movement of the heart was described as peristaltic and was likened to that seen in portions of the alimentary tract. Guided by observations upon cold-blooded vertebrates, physiologists regarded the movement as a muscular wave, originating in the neighbourhood of the sinus venosus and passing over the chambers of the heart in regular order. But when it became known that the system of the ventricular contractions may in certain circumstances become independent of the auricular contractions this view was largely abandoned.

In the middle of the last century collections of ganglionic nerve cells were

discovered in the auricular wall of the amphibious heart; the functions of these cells were not understood, they were for many years wrongly interpreted. The incorrect interpretation obtained a powerful hold upon men's minds, colouring their thoughts and their observations. The cells were regarded as central regulating stations, from which the rhythm of the heart was propagated, from which the sequence of chamber contraction was ordered. It is to this period that the observations of Stannius belong. Though doubts arose at a later date, it was not until W. H. Gaskell published his observations (9), that the hypothesis of a neurogenic control of sequence in the sense in which it was then employed was laid finally to rest. The discovery of Gaskell which now concerns us followed and was prompted by the work of that distinguished English scientist Romanes, upon the contractile bell of jelly fishes. Romanes (20) proved that the contraction is propagated as a wave through the bell and that the direction of its spread is governed by continuity of the tissue. The wave of contraction could be diverted by systematic incisions, zig-zag or spiral as the case might be, and would follow regularly the path devised for it, however unusual such a path. Gaskell demonstrated the same fact in the muscular wall of the auricle, proving beyond cavil that contraction travels as a wave, and that the contraction of one segment of an isolated strip is provoked by the passage of the contraction wave into it from a neighbouring segment. He showed, as Romanes had done for the umbrella of Medusa, that the sequence of contraction in the elements of a strip of heart muscle is independent of the natural order in which these elements contract, that continuity alone guides the flow of the wave. He showed that the muscle of the heart may be so cut as to disorganise the supposed system of governing nerve fibres united to a central nerve station without interrupting the passage of the wave. He clearly enunciated his conclusion that the passage of a contraction wave from one mass of heart muscle to another depends upon the bridging of the gap between them by muscle tissue and upon the functional integrity of the bridge. He demonstrated that when one mass of heart muscle contracts in sequence to another, the stimulus which promotes the contraction of the former is derived from the activity displayed by the latter. If the functional integrity of the bridge is impaired by such an experimental procedure as pressure upon it, then the response in the distal mass is delayed, or interrupted, according to the degree of damage. These and other ingenious experiments overthrew those hypotheses which held the nerve cells in high relief, and gave the lead to the work which followed; for it was assumed, in the light of his experiments, that the contraction travels throughout the heart as a wave from one chamber to the next and that it is carried across the gaps by means of muscular

bridges. As a sequel the attention of workers gradually became focussed to discover (1) the precise point at which the contraction originates and (2) the precise paths followed by the natural wave as it travels over the heart.

I do not propose to follow in detail the growth of knowledge from the time of Gaskell's experiments; I shall be content to enumerate the chief steps as they were taken; neither shall I follow those steps in their exact chronological order. Gaskell worked upon the relatively simple hearts of the frog and the tortoise; my own object as a clinician has been an understanding of the motion of the mammalian organ; to this, therefore, I shall now in the main confine myself.

For several reasons, investigation of the mammalian heart is more difficult than that of the cold-blooded heart. The mammalian organ is more viable, it is more complex, its movements are far more rapid. The blood stream through the mammalian heart has to be maintained, the heart insists upon respectful treatment, otherwise the natural beat is not maintained. I lay particular emphasis upon the fact that those methods of investigating the mammalian heart which subject the organ to the least manipulation and damage are the methods which are most successful in elucidating the nature of its beating. The rapidity with which the contraction wave passes over the tissues of the mammalian heart necessitates the use of delicate apparatus in its study. Our methods of registering the movement in different heart chambers have improved very rapidly of recent years; systems of recording levers have become ever lighter and quicker in their movement, but mechanical contrivances, much as they have been employed for the purpose, have so far proved inadequate. For accurate observation, electrical methods have almost wholly replaced them. In 1878 the first records of the heart beat were taken by Burdon-Sanderson and Page (21), using the capillary electrometer; their work upon the tortoise heart may be regarded as the real starting point of modern electrocardiography. Five years later Waller (24) showed that the beat of the heart may be recorded in mammals (including the human subject), without exposing the organ, without damaging the animal in any way. In 1892 the mammalian heart was the subject of special study by Bayliss and Starling (1). These early galvanometric studies, while throwing little actual light upon the course of the contraction wave, are, nevertheless, to be regarded as essential steps; they opened up a new pathway, which later workers have pursued; in this sense the workers were pioneers. It was in the present century that Einthoven (4), in Holland, perfected an instrument which, on account of the facility of its working and the precision of its movements, has enabled us to unveil much which was formerly mysterious.

This instrument is the string galvanometer, and its construction must rank as a chief milestone in the study of the questions which we are considering. It has been known for very many years that, when muscle becomes active, that is to say, when it passes to a state immediately premonitory to the actual contraction, it becomes relatively negative to inactive muscle, in the sense that the zinc element of a copper-zinc couple is relatively negative to the copper element. When a wave of contraction passes through a strip of muscle, it travels in the immediate wake of what is termed the excitation wave, a wave of electrical change whose crest is a crest of relative negativity. Each part of the tissue, as it is about to become involved in the contraction process, shows this electrical change; the moment at which each part of the tissue is about to contract can be signalled by a sufficiently delicate instrument. It was this fact and Einthoven's instrument of which I took advantage when I commenced my studies of the origin and propagation of the contraction wave throughout the heart.

From this general historical introduction, we may proceed to examine the chief problems which presented themselves, and which have now been solved.

The Pacemaker of the Mammalian Heart and the Spread of the Excitation Wave in the Auricle.

In the cold-blooded heart the contraction wave has been recognised long since to start in the region of the mouths of the great veins which enter the sinus venosus, for in the frog and the tortoise the sinus contracts first, and is followed by contraction of the auricle. This sequence may be witnessed in the frog or tortoise with the unaided senses; it is disturbed if a clamp is placed upon the sinus, for then, while the mouths of the veins continue to beat at their old rate, the whole of the auricle and ventricle remains quiescent.

In the mammalian heart an anatomical sinus does not exist, sinus and auricle have become closely incorporated; but morphological reasoning directed attention to the mouths of the great veins, to the superior and inferior vena cava, as the probable starting point of the contraction wave. A number of early experiments, conducted for the most part upon the dying heart or upon the heart mutilated by fatal incisions, purported to demonstrate that the heart beat starts in the region of the great veins; this localisation lacked both precision and certainty. A chief step (12) was taken when Keith and Flack (1907), in their search for sinus remnants, lighted upon the highly differentiated mass of neuro-muscular tissue, which has since been termed the *sino-auricular node*. This mass of tissue lies in the dog immediately to the caval side of the *sulcus terminalis*, a line bounding

the mouths of the two cavæ; it extends along the ventral border of the superior cava for some distance. This discovery was significant and prompted the experiment which I am about to describe. Simultaneously with the observations of my laboratory, Wybauw, in Liége, instituted independent observations (14 and 26). The results of these investigations were in perfect harmony; we speedily found that the region of the sino-auricular node becomes relatively negative before any other point on the surface of the auricle. The method first adopted was relatively crude; we sought the direction of the first flow of current produced by the action of the auricle through the galvanometer, when leading off from pairs of chosen points on the auricular surface. It was shown that, providing one contact lies over the sino-auricular node, it is a matter of indifference where the second contact is placed, the *S-A* contact is always negative at the start of the electric change. These observations have since been abundantly confirmed by similar observations and by the method of cooling. The last method merits further description. A withdrawal of heat from the tissues is well known to depress their functions, and in experiments upon the frog's heart, cooling of the sinus region was known to decrease the rate of the heart's beating. It does so because it depresses the function of the tissue elements in which the heart's rhythm has its origin. MacWilliam (19) and Flack (7) were responsible for some of the earliest experiments on cooling in the mammalian heart. The rate of the mammalian heart beat is lowered when the area containing the sino-auricular node is cooled, and this reduction of rate is provoked by cooling no other region of the heart (8). This experiment has confirmed in a striking manner what we already knew, namely, that the wave of contraction starts in this locality, but it has shown further that the impulses which promote the rhythm are born there.

At a later date a method (16) was devised in my laboratory whereby the time at which the excitation wave reaches any given point on the surface or lining of the auricle can be determined with a maximal error of 0·002 second. This method allowed us to map out in a precise fashion the wave of excitation in the auricular musculature over the greater part of its course. Our conclusions may be briefly summarised. The excitation wave starts in the head or swollen part of the sino-auricular node; it spreads from this node at an average rate of 1000 mm. per second into the surrounding auricle along lines radiating in every direction from the node. It is noteworthy that the surrounding muscle is arranged in bands which converge in this region of the heart as though arranged to speed the spread. I have likened the spread in the auricle to the spread of a viscous fluid poured from a funnel upon an almost flat surface; the margin of the fluid invades the plate as an ever

widening circle. The spread in the auricle differs in this respect only, that it is confined to the muscle bands. The wave spreads up the superior cava against the blood stream; when it reaches the mouths of the inferior cava and of the pulmonary veins, it similarly spreads up them; it spreads from the base to the apex of each auricular appendage; it spreads down the septum towards the ventricle. There are no special paths of conduction; the spread is uniform and from one muscle element to contiguous ones, and involves the two auricles as though they composed a single sheet of muscle.* The spread in the auricle is ordered upon a simple plan.

Spread from Auricle to Ventricle.

Between the contraction of the auricle and the ventricle there is considerable delay; the delay is very pronounced in the frog and tortoise. It was Gaskell who showed, by placing a clamp upon the groove which separates these two chambers and by gradually tightening it, that precisely the same disturbance of sequence may be induced as when a clamp is applied to an isolated strip of auricular muscle. By clamping, the passage of the wave is at first hindered, later the ventricle fails to respond after occasional auricular contractions, later still as pressure is increased the responses of the ventricle become fewer until there is no response at all. These experiments led Gaskell to the conclusion that the sequence of the ventricular upon the auricular beat can be explained without the intervention of any special nervous mechanism, that the passage of the impulse over the groove is of the same nature as its passage over a bridge of tissue in the incised auricle. He showed also that this sequence is not disturbed by removal of Bidder's ganglia. In the frog the junction between auricle and ventricle is formed by a ring of tissue, and composed in the main of muscle fibres of relatively primitive type. This primitive muscle formed, in Gaskell's view, the functional bridge between the two chambers. Following Gaskell's studies came the early experiments of Tigerstedt (23), and of Wooldridge (25) and MacWilliam (19) in this country. Their experiments determined that conduction across the *A-V* groove in the mammal is subject to the same disturbances as is conduction in the frog, and showed that the impulse received by the ventricle is not the filling with blood by the auricle, and that the impulse is not conveyed through the superficial cardiac nerves. But at this time and for many years afterwards the auricle and ventricle of the mammalian heart were not known to be joined by a bridge of muscle

* In this conclusion we differ from Eyster and Meek (6), who believe that there is specially rapid conduction to the *A-V* node. Our criticisms of their methods have been published (16).

tissue, though nerves pass freely over the auriculo-ventricular groove. The musculatures of the two chambers were, in fact, widely believed to be disunited. It seemed as if either Gaskell's conclusions for the cold-blooded heart lacked finality, or that a fundamental difference exists between the mechanism in frog and mammal. The obvious discrepancy was removed from the minds of English physiologists by Kent's description of a muscle union between the two chambers in the mammal in 1892 (13). A year later, His described the muscle bridge more distinctly, and made the first experiments upon it (11). The final experiments were made by the Americans, Erlanger (5) and Cohn (3), and others (10). It has been proved beyond all reasonable doubt that this muscle bridge, which runs from the auricular to the ventricular septum, is in the mammalian heart the sole path by which impulses are conveyed from auricle to ventricle. That pressure upon or cooling of this bundle hinders the passage of the impulse, that division of this bundle completely dissociates the movements of auricle and ventricle, is now recognised; these effects are regularly obtained in a number of experimental laboratories.

Distribution of the Wave in the Ventricle.

I pass over the earliest experiments upon the spread of the contraction wave through the ventricle. The observations were electrical, and were inaugurated by Burdon Sanderson; they were contradictory because the complexity of the spread was not appreciated.

In respect of the spread in the mammalian ventricle a great step in the progress of knowledge came with the anatomical discovery of Tawara (22), the Japanese. Many years before Purkinje had described a network of highly differentiated cells lying beneath the endocardium in mammals. The functions of these cells were in his time quite unknown. Tawara demonstrated that they compose in each ventricle a striking basketwork, lining each chamber. He traced the ending of the muscular bundle which unites auricle and ventricle into a right and a left division, each of which by a few chief strands unites with the basketwork lining the corresponding ventricle. He concluded, and rightly concluded as it transpires, that the bundle forms with its branches and arborisations the channels by which the impulse to the mammalian ventricle is distributed. The experimental proof that this is so is now forthcoming.

The ventricle is the chamber which accomplishes the heart's real work; it is powerful muscle, arranged around the blood-containing cavities, and forming to these cavities a thick wall. The muscle fibres are arranged in an intricate fashion, largely in the form of broad spiral bands, some of

which encircle both cavities, while others limit themselves to a single cavity. The chief spiral bands start at the bases of the ventricles and pass to the apices of the ventricles where, turning sharply, they form vortices and pass up to constitute the papillary muscles. The arrangement is such that these layers may be dissected off one by one, and as each new layer is reached, the inclination of the fibres alters.

Our first efforts (17) to unravel the course of the excitation wave took account of these muscle bands, for we naturally supposed the course of the wave to be controlled by them. But the readings which we obtained demonstrated to us clearly that the times at which the excitation wave appears at different points of given superficial bands are incompatible with this view. We explored the whole superficies of the ventricle, and constructed maps of the ventricle in a number of animals which showed the precise surface distribution of the excitation wave in relation to time. These maps portrayed a number of new facts. It was to be seen that the system of distribution is tolerably uniform from animal to animal; it was shown, considering each ventricle separately or the two ventricles together, that points widely apart are activated simultaneously. A simple form of distribution, the passage of the wave from one muscular element to the next, a radial spread from a given point on each ventricle or a given area of each ventricle would not suffice; we were compelled to recognise that the spread occurs to a large number of surface points simultaneously; no other hypothesis could explain the rapidity of the spread or the order of it; we were forced to the conclusion that the excitation wave is distributed by a number of separate and distinct channels. This conclusion was at variance with the conclusions of workers who had previously used the electrical method: these had all assumed a simple form of spread; but it harmonised in a general way with the recent anatomical discovery of the arborisations of the *A-V* bundle. Attention was consequently directed to this system, and experiments specially devised to test the matter proved its importance.

If after mapping out the distribution of the wave to the ventral surface of the two ventricles, the right division of the auriculo-ventricular bundle, which breaks up into the network of the right ventricle, is divided, the spread becomes altered (17 and 18). Over the left ventricle it remains unchanged, over the right ventricle it is delayed; moreover, after such a lesion, the order in which the surface is activated is found changed; the wave now starts at the margin of the right ventricle where it borders the left ventricle and travels over the ventricle away from this margin. Briefly, when the right division of the bundle is cut, the excitation wave at first spreads only to the left ventricle; later, when the left ventricle is

completely involved, the right ventricle receives the wave through the muscle which unites the two chambers; thus, after section of the right stem of the bundle the right ventricle is activated in a high abnormal manner.

Another experiment explores the network itself. The natural spread of the excitation wave over the conus of the right ventricle is away from the chamber of the ventricle and towards the outlet of the pulmonary artery. A cut traversing the conus at right angles to the general direction of spread and penetrating the muscle fibres for a considerable depth, fails to delay the appearance of the excitation wave above the incision, while a shallow incision or even a scratch in a similar plane, but applied to the inner or endocardial surface, produces a profound delay. Normal conduction is therefore through the branches of the auriculo-ventricular bundle and ultimately through the arborisation and network.

But this demonstration still leaves several questions unsolved. If the impulse which descends the bundle from the auricle is distributed to the networks lining the cavity of the heart, by what paths is it conveyed to the surface of the heart? It is conveyed, as the following experiment clearly shows, by direct penetration of all the muscle layers along paths radiating from the cavity. The muscle of the left ventricle is very thick, and can be deeply incised with impunity; if a point is chosen on the surface, and the time at which the excitation wave arrives at it is determined, this time is uninfluenced by deep incisions which completely surround the point in question. But if an incision is made which undercuts the point examined, the wave is greatly delayed in its passage. This simple experiment shows that the wave is carried from within outward from one muscle band to the overlying one, and that it travels in a plane which is at right angles to the direction of the fibres. In support of the same conclusion are time readings taken from the cavity of the heart; if corresponding points on the inside and outside of the heart are tested, it is found that the excitation wave always reaches the lining before it reaches the surface of the heart.

Now the facts which I have so far related point steadily to one conclusion, namely, that the distribution of the excitation wave in the mammalian heart is accomplished through the branches of Tawara's tree. Arriving at the bundle the impulse travels through the main branches, through the smaller branches and twigs, until the whole lining of the cavity becomes implicated; from the network which lines the cavities it spreads directly into the ventricular wall, penetrating each layer of it in succession. This general account appears a simple and satisfactory explanation of the facts so

far as I have related them, but the problems which arose during the researches were not so simple; an observation, of the soundness of which we were convinced, for a long while mystified us. Our surface maps showed that the earliest parts of the surface to be activated are the extreme apex of the left ventricle and an area of the right ventricle lying a little above its apex. So far as the latter was concerned there was no great difficulty, for in the dog, upon which our experiments were conducted, this region of the right ventricle is directly supplied by large branches of the arborisation which pass directly to it. No such direct strands pass to the apex of the left ventricle; moreover the activation of the surface of the left ventricle at a little distance from the apex, although this surface lies nearer to the trunk of the tree than does the actual apex, is activated almost as late as any part of the heart's surface. This discrepancy is one of many observed; the surface distribution is not to be explained solely by the length of conducting fibres which pass to underlying points of the lining; there is some other and chief factor influencing the surface distribution. This factor was discovered when the rates of conduction of the excitation wave propagated by electrical stimulation were investigated. If two points, one of which lies on the surface and the other of which lies on a corresponding part of the lining, are tested and readings are obtained from them while the heart is beating naturally, the point on the lining is the first to show activity; this is natural, seeing that the impulse has its starting point within. But if the same points are tested when the excitation wave is artifically excited from a *point on the surface* at some little distance from the tested points, *the same phenomenon is witnessed.* The point on the lining becomes active first, and by the same time interval, as it does when the heart is beating naturally. The excitation wave reaches the point on the lining first, although that point is further away from the point of stimulation than is the point on the heart's surface. It now began to be evident that conduction along the lining is more rapid than is conduction along the surface. That the rapidity of conduction in the lining is due to the Purkinje network is shown by scratching the lining between the points tested and the stimulating electrodes; after this interference the point on the lining is no longer activated first. On the other hand a deep incision into the surface muscle, though almost penetrating to the cavity, does not affect the time relations. Further investigations showed that the interval which elapses between the arrival of the wave at the points tested varies within certain limits according to their distance from the point of stimulation. If this distance is reduced sufficiently, then the excitation wave arrives at both points simultaneously. By suitably arranged experiments and measurements of the network and thickness of the muscle walls it is possible to calculate

the ratio between the rate of conduction through the muscle and through Purkinje tissue. The proportion is approximately as 1 is to 10.

Again, if the rate of conduction between two points on the surface of the heart is examined and these points lie on the wall of the ventricle where this is thickest, the conduction rate is approximately 300–500 mm. per second; a similar rate of propagation is ascertained during the natural passage of the wave from within outwards through the muscle wall; but if the rate of conduction is tested similarly over thin portions of the ventricular wall, it is found to be as high as 1500–2000 mm. per second. In the last-named circumstances the wave is short-circuited through the Purkinje network for the longest part of its course. After consideration of all the circumstances we have concluded that the rate of conduction through ventricular muscle lies between 300–500 mm. per second, while that through straight paths of Purkinje tissue lies between 3000–5000 mm. per second.

This conclusion clears away the discrepancies in surface readings to which allusion has been made. The surface points which are activated earliest are those points which overlie the thinnest parts of the ventricular walls. There is an unmistakeable relation between any surface reading and the thickness of the muscle at the point tested.

The surface readings are controlled by two factors: first by the length of the Purkinje strands and secondly by the thickness of the muscle wall. If a number of surface points are tested, and the heart fixed; if measurements are made upon the lining and on the basis of the estimated conduction rate over the lining the corresponding times are calculated; and if measurements are made of the thickness of the ventricular wall and on the basis of the estimated conduction rate in ventricular muscle the corresponding times are calculated and added to those of the first series; then a complete series of calculated readings is obtained which harmonises with the readings won from the naturally beating heart.

A General Summary.

We are now in possession of a very detailed knowledge of the course taken by the contraction wave through the mammalian heart. It behoves us, in so far as we are able, to review the mechanism of the heart beat in the light of this new knowledge. The mammalian heart has onerous duties to perform, but the first plan of its construction is the plan of a simple tube contracting peristaltically from end to end without interruption. During phylogenetic and embryological development the work of the heart increases, the shock of its movements increases. The heart divides in two, an upper and lower chamber, the first frail and thin-walled, the second sturdy and

massive. Why are these two chambers developed in the heart? The function of the auricle is not to fill the ventricle; the blood pumped from auricle to ventricle at the end of the heart's diastole is but a fraction of its full content. The function of the auricle is to save the veins from over-distension. During a third of the ventricular cycle, during the period when the ventricle is pouring blood into the body, the circulation is completely obstructed at the auriculo-ventricular orifices. Yet the blood flow in the veins continues; the flow goes on into the expanding auricles. The mechanical function of the auricle is that of a reservoir, to catch and hold the content of the stream when a dam is thrown across it. It wants little strength for this task; its walls must stretch easily; they have to pump against a low resistance; the muscle layer is therefore thin.

The first sign of contraction is in the region of the opening of the superior cava; it quickly spreads to the mouth of the inferior cava. The mouths of these veins tend to close before the main mass of auricular muscle is involved, thus hindering or preventing regurgitation. The spread of the contraction wave to the auricular muscle as a whole is expedited by the central position of the node from which the wave starts, by the architecture of the muscle bands which radiate from it in all directions, and, lastly, by the relatively high rate of conduction which auricular muscle possesses (1000 mm. per second). In the mammalian auricle the plan of distribution is primitive.

The wave travels through the auricle to the first part of that bridge which joins the auricle and ventricle. Here there is delay and long delay, while the auricle is emptying itself and the auriculo-ventricular valves float up preparatory to closing. This delay occurs, so it is believed, in the auriculo-ventricular node, a structure of very small muscle fibres (15). Leaving the node, the wave passes to the bundle. The need of this structure and of its branches and twigs has developed with the division of the ventricle into two cavities. This special system of fibres is endowed with the highest order of conducting power; it is arranged that both ventricles and all their parts may be thrown into contraction with the closest approach to simultaneity; in that lies a mechanical advantage which the ventricle as a driving muscle demands for itself. The wave does not travel along the ventricular wall: were that so and were its progress as rapid as in the wall of the auricle, its course, nevertheless, would be slow. It penetrates the wall; its course in muscle is short, and therefore a high rate of conduction in the muscle is not essential; we actually find it to be of a low order.

The system of distribution in the thick-walled ventricle possesses a further virtue in that it protects the laminated tissues from disruption.

A contraction wave following the spiral muscle bands, bands arranged to wring the blood powerfully from the heart, would tear them asunder, would rupture the fragile vessels penetrating all at right angles.

Gaskell, with that acuity of perception which was his pronounced quality, thought of the muscles of the sinus and of auriculo-ventricular ring as primitive remnants, highly endowed as is the primitive cardiac tube with the function of rhythmicity, poorly endowed with the function of conductivity; he associated the fine structure of the tissue of the rings with peculiarity of function. A similar but more complex relation between structure and function is to be demonstrated in the striated tissues of the mammalian heart. Rhythmicity as a function is most highly developed in the sino-auricular and auriculo-ventricular nodes, structures which closely resemble each other in the fine details of their construction, structures which are now both held to represent remnants of, or developments of, the original sino-auricular ring. Conductivity is a function held in varying degree by the mammalian tissues. The Purkinje cells are the largest striated cells, and possess the highest content of the carbohydrate glycogen; they conduct most rapidly. The fibres of the auriculo-ventricular node, the smallest to be found in the heart, conduct most slowly (15); they are almost devoid of glycogen. Between these two extremes are the fibres which compose the walls of the auricle and ventricle. These fibres are of intermediate size, the content of glycogen is intermediate, the power of conduction is intermediate. The ventricular fibres are said to contain less glycogen than the auricular (2), they also conduct less rapidly. Thus, the rate at which the wave flows is controlled by the structure and chemical constitution of the tissues through which it passes; the musculature in its various parts is so differentiated that from its appearance the manner of its working may be known.

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On the Influence of Vibrations upon the Form of Certain Sponge-Spicules.

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It has been pointed out recently* by one of us that the development of the remarkable chessman-spicule or discorhabd in the genus *Latrunculia* is a somewhat complicated process depending upon several factors. The protorhabd or axial thread appears first as a slender rod capable of independent growth. With these protorhabds two kinds of silica-secreting cells appear to be associated, viz., formative cells which are responsible for the actual deposition of the silica upon the protorhabd, and accessory silicoblasts which are supposed to collect supplies of silica and bring them to the formative cells to be used in the process of spicule-formation.* The spicule in this case consists of an elongated axis with whorls of flattened lobes arranged at more or less definite intervals along its length, and it was suggested that the

* A. Dendy, Presidential Address to the Quekett Microscopical Club, 'Journ. Q.M.C., Ser. II, vol. 13, p. 231 (1917).

position of these whorls is determined by the fact that the spicule, at the time of their commencement, is in a state of vibration, due to the water currents flowing through the sponge, the whorls corresponding to the nodes or positions of comparative rest. The special accumulation of silica on the nodes appears to be due, not directly to the vibrations of the spicule, but to the fact that the formative cells exhibit a kind of tropism which induces them to settle down and perform their work in the positions where they are least disturbed by the vibrations.

The whorls in this case are not sharply defined at the moment of their first appearance, so that it is impossible to obtain accurate measurements for mathematical analysis; nevertheless, there are certain facts connected with their arrangement which, in our opinion, afford a fairly conclusive demonstration of the view that they are deposited approximately upon the nodes of a vibrating rod. Two species were investigated, *Latrunculia apicalis* and *L. bocagei*. In both species the spicule, at a certain stage of its development, consists of a straight rod with four thickenings, representing a basal manubrium and three incipient whorls. There is a basal thickening at one end, an apical thickening at the other, a median thickening at or near the centre, and a subsidiary thickening, usually between the median and apical thickenings, but occasionally between the median and basal thickenings. If these thickenings correspond to nodes, we have to account for the fact that a subsidiary thickening is developed only on one side of the median thickening. The solution of this difficulty is to be found in the arrangement of the formative cells (observed in *Latrunculia bocagei* only, though doubtless occurring in the other species also), for while there is a ring of formative cells round the median thickening and a similar ring round the subsidiary thickening, there is none around the part of the spicule where a second subsidiary thickening might be looked for, and hence no whorl is developed in this situation, in spite of its being a nodal point. No formative cells have yet been observed in relation to the basal and apical thickenings.

Certain differences in the development of the discorhabd in the two species of *Latrunculia* dealt with are of the highest significance from the point of view of the vibratory theory. In *L. apicalis* the young spicule is symmetrical, the basal and apical thickenings being represented by rounded knobs of approximately equal size, while the median thickening, consisting typically of a whorl of three knobs, appears just about half-way between them, developing either simultaneously or (perhaps always) a little later. In *L. bocagei*, on the other hand, the apical thickening appears some time before the basal thickening, so that we have an asymmetrical rod weighted at one end, and the median thickening is actually shifted towards the weighted end

exactly as might be expected if it really indicates the position of a node. Of course individual variations occur, due, doubtless, to various disturbing factors, but these do not invalidate the general conclusion that the thickenings represent nodal points.

As already stated, the whorls of the *Latrunculia* spicule are not sufficiently well defined or regular on their first appearance to yield accurate measurements of their position. There is, however, amongst the remarkable collection of sponges obtained by the "Sealark" Expedition in the Indian Ocean, a species belonging to a new genus related to *Latrunculia*, in which a discorhabd occurs which is much better suited for mathematical investigation. As the species has not yet been described, we may refer to this spicule simply as the oxydiscorhabd, from the fact that it is an oxeote spicule with two disc-like whorls. The shaft of the spicule (fig. 15) is thickest in the middle, and tapers gradually to a sharp point at each end, being approximately symmetrical but slightly curved. The young spicule (figs. 4–14) may be slightly angulated in the centre and, as is frequently the case in oxeote spicules, may exhibit a slight central enlargement (the primary central thickening), which, however, as will appear later on, has nothing whatever to do with the formation of a whorl. When fully developed, the surface of the shaft is slightly roughened with numerous small points. The spicule evidently belongs to the common diactinal type, in which two of the rays of the primitive tetract have been suppressed, and, before the formation of the whorls (fig. 4), it is doubtful whether it could be distinguished from the young oxeote megasclere of the same sponge. The whorls, when fully developed, are thin discs of silica (fig. 15), not divided into lobes, but with an irregular margin; the median one is concave on the side facing the other, which latter evidently corresponds to the subsidiary whorl of the *Latrunculia* spicule.

Unfortunately the only specimen in which this oxydiscorhabd occurs is not sufficiently well preserved to enable us to say anything about the cells which are concerned in the development of the spicule, but it seems reasonable to assume the existence of formative cells having the same relation to the whorls as in *Latrunculia bocagei*.

Only two whorls are developed, and these do not appear until the shaft has attained a considerable thickness, measuring about 0·066 mm. in length by 0·0023 mm. in diameter in the middle. Then the whorls appear, almost, if not quite, simultaneously, the median one at or very near the centre of the shaft, and the subsidiary one at one side of it, always nearer to the centre than to the end. At first, each whorl is represented by a thin, sharply defined ring of silica, the position of which can be accurately determined by measurement.

Since the spicule continues to grow after the whorls have appeared, it is obvious that the only measurements of any value for our purposes are those made upon spicules on which the whorls are only just commencing their development. We may call this the critical stage. After it has passed, the distance between the two whorls will not increase, but the distance between the subsidiary whorl and the end of the spicule increases considerably. It will be observed that, as in the case of the *Latrunculia* spicule, only one subsidiary whorl is formed, and we may attribute the absence of a second one to the same cause, viz., the absence of the necessary formative cells.

Before proceeding to discuss the position of the whorls, it is desirable to say something about the way in which the measurements were made. The spicules were first drawn with a camera lucida, under a Zeiss microscope, with an F objective and a No. 4 eyepiece, giving a magnification of about 1075 diameters. The outline was corrected without the camera, and measurements were made upon the finished drawing. In making the measurements and calculations, the absolute dimensions of the drawing, and not those of the spicule itself, have been employed.

It will be observed that the case is a different one from that of the *Latrunculia* spicule, for the ends of the rod are not weighted, and the vibrations must be those of the free-free type. It is safe to assume that any vibrations that occur must be transverse ones, for, whereas it is easy to see how transverse vibrations might be induced by sudden alterations of pressure upon a slender elastic rod lying in the almost liquid mesogloea in the immediate neighbourhood of the canals through which water is flowing, it is extremely improbable that vibrations of any other type would be set up.

The positions of the nodes in a transversely vibrating rod of uniform section are, of course, well known, but, apparently, no investigator has hitherto determined, either by experiment or calculation, the corresponding positions in the case of a rod having the form of a double cone or paraboloid, or some form intermediate between these two, such as is exhibited by the oxydiscorhabd. It was therefore necessary in the first instance to arrive at a general formula, by means of which this problem could be solved, and then to calculate the theoretical positions of the nodes in each spicule examined. The degree of accuracy with which the positions found by actual measurement agree with those determined by calculation, based upon the form of the spicule, affords the best possible test of the truth of the vibratory theory.

The lateral vibrations of a bar of uniform cross-section and material are investigated very fully in Lord Rayleigh's treatise,* and we may quote, for

* 'Theory of Sound,' vol. 1, p. 256.

reference, the results which are of interest from the present point of view. These relate, of course, entirely to the positions of the nodes. When the bar is set in vibration, the various tones are not excited equally strongly, and the nodes which may be expected to occur are only those associated with the two gravest vibrations—or the more fundamental vibrations. Any higher vibration is of necessity accompanied by graver notes, which cause the nodes of the higher vibration to be in motion, so that their significance as points of rest must be diminished or destroyed. If the length of the bar be taken for convenience as unity, and if it be free at both ends, the most fundamental vibration gives nodes at distances 0·2242 from either end, while the second tone has a node in the middle and two others at distances 0·1521 from either end. The first tone is of much greater importance, and the usual vibration must consist mainly of a superposition of the first and second tones.

If, on the other hand, the bar is fixed at one end and free at the other, the fixed end must be a node. The second tone gives a node at 0·2261 from the free end, and the third gives a node at 0·1321 from the free end, and another in the middle of the bar. It is not necessary to consider, for the particular type of spicule dealt with in the present communication, the case of a bar fixed at both ends.

When attention is restricted to the most important vibrations, we see that the free-free bar gives nodes at 0·2242 from each end and one in the centre (which is common to many tones), while the fixed-free bar gives a node at 0·2261 from the free end. These distances are practically identical. The fixed end in the latter case is also a node.

This result is not directly applicable, in the quantitative sense, to the spicule with which this communication is concerned, but, at the same time, valuable qualitative information can be derived at once in general terms. If a bar is thick in the middle and tapers to a point at each end, it may be regarded in two ways. In the first place, if it be looked upon as a free-free bar symmetrical about its centre, the nodes, ordinarily at 0·2242 from each end, must move inwards towards the more inert centre, in accordance with a general principle of vibratory motion. The bar may, however, if the centre is very thick in comparison with the ends, and therefore almost entirely free from vibratory motion, be regarded as made up of two fixed-free bars placed together but vibrating independently. There must then be a node at the centre, and two others, one for each bar, at a greater distance than 0·2261 from the free end. The general conclusion, from both points of view, is that a node should be expected at the centre, and two others, one on either side, whose distance from the corresponding free end cannot be so small as 0·2242.

Another question of some importance, to which a preliminary statement may now be devoted, is that of curvature. The well known experiments of Chladni showed that the effect of bending a bar, so that it begins to approximate to a tuning fork, is to make the nodes of the vibrations approach the point of maximum curvature in the centre. Thus the two nodes of the fundamental vibration of a free-free bar, originally at a distance of 0·224 from its ends, move inwards towards the centre as the bar is bent. A diagram given by Chladni, showing the rate of approach of these nodes, is reproduced by Barton.* The spicule with which we are concerned is somewhat bent, and this result is therefore of importance. The bending is, however, so slight that the shift of the nodes due to this agency may be regarded as additive to that produced by inequalities in the cross-section, and it is not necessary to obtain a quantitative solution of the very difficult problem of a bent bar of non-uniform cross-section.

The lateral vibrations of bars of variable cross-section have not been investigated hitherto, in the strict quantitative sense, as regards the positions of the nodes, in any case; although Kirchhoff† determined the periods of vibration of a bar which was in the form of a thin cone, and of another bar which has no definite relation to the present problem. A mathematical solution of the complete problem for several cases has, however, been obtained by one of us for the purposes of the present investigation. Full details of these results are contained in another paper communicated to the Society. They include the case—important from its approximation to that of the spicule treated in detail here—of a bar which consists effectively of two equal thin cones with their flat ends in contact, as in fig. 1. If $2l$ be the length of such a bar, and q a multiplier, dependent on

FIG. 1.

the period, it can be shown that the primary mode in which the bar vibrates symmetrically, so that the curvature of the axis at some particular instant is of the form given in fig. 2, is determined by the equation $J_3[2\sqrt{(ql)}] = 0$, and the nodes are at a distance x from either free end determined by $J_2[2\sqrt{(qx)}] = 0$. The J's represent Bessel functions, and the roots of these equations are already known from their occurrence in other physical

* 'Text Book on Sound,' p. 296.

† 'Berlin Monatsber.,' 1879, pp. 815–828.

problems.* Taking the first root in each case as corresponding to the fundamental vibration of symmetrical type, we find

$$2\sqrt{(ql)} = 6{\cdot}379, \qquad 2\sqrt{(qx)} = 5{\cdot}135,$$

and therefore $$x/l = 0{\cdot}648,$$

or, the distance of the node from the free end bears to the whole length of the rod the ratio 0·324, as against 0·224 for a uniform rod.

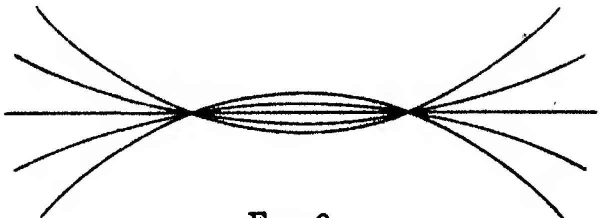


FIG. 2.

Each of the unsymmetrical vibrations has one node at the centre of the rod. This is evident without analysis. When such a structure vibrates, therefore, the nodes which should be prominent, as corresponding to the more fundamental and therefore stronger vibrations, are three in number—one at the centre and one at 0·324 of the length measured from each end.

The uniform rod and double cone are limiting cases. When the rod is not bent, the nodes for any intermediate configuration can be approximately predicted, but must be between the limits 0·224 and 0·324. When the rod is sharply conical the second value should be nearly reached. The corresponding value for a nearly double-parabolic rod, as in fig. 3, is 0·29. Bending of the axis of any rod must always make these theoretical values higher by pulling the nodes in towards the centre.

FIG. 3.

Quoting a more general result of the mathematical investigation above, we may state that, if a bar is composed of two equal halves, each consisting of a portion of the solid formed by rotating the curve $y = Ax^n$ about the axis of x—in practice the axis of the spicule—the distance of the symmetrical node from the free end is the product of the length and a numerical factor

$$0{\cdot}074 + 0{\cdot}300\,(4n+1)/(4n+2)$$

with great accuracy.

* Lord Rayleigh, 'Theory of Sound,' vol. 1, p. 330.

For any suitable spicule the value of n may be determined by direct measurement of the varying transverse diameter in the magnified drawing. For this purpose, it is essential, as already stated, to obtain specimens which have just reached the critical stage (when the whorls are only commencing to develop). In such cases very accurate measurements are possible. Moreover, progressive changes in the position of the actual nodes, caused by the fact that the more developed spicule may not be exactly similar to its original shape when the whorls commenced to form, cease in these circumstances to be a disturbing factor. The fact that the whorls on the oxydiscorhabd appear first as very thin, sharply defined rings, makes it possible also to determine their position with considerable accuracy.

An account is given below of the quantitative examination of ten specimens of the oxydiscorhabd, at or near its critical stage of development, from the standpoint of the vibratory theory. It is necessary, in view of the preceding account of the biological history of the spicule, merely to show that the positions of the two actually occurring whorls are in accordance with the theory of vibrations of non-uniform bars already outlined. The absence of the third whorl has already been explained.

The magnification adopted for each of the specimens was, as already stated, about 1075, and the measurements were all made upon the enlarged drawings, the actual dimensions of which will alone be quoted.

If the curve of any spicule is of the form

$$y = \mathrm{A}x^n,$$

and if $2y_1$, $2y_2$ are breadths at distances x_1, x_2 from the end,

$$2y_1/2y_2 = (x_1/x_2)^n$$

or

$$n = (\log 2y_1 - \log 2y_2)/(\log x_1 - \log x_2).$$

A series of values can be found for n by such measurements and a mean value taken. For example, for the first of the ten spicules (fig. 5), measurements are shown in the following Table:—

Breadth ($2y$).	Distance from end (x).
mm.	mm.
2·6	84
2·3	27
2·1	20·5
1·5	9

From the third and fourth pairs of observations of position and breadth quoted in the Table

$$n = \frac{\log 2\cdot 1 - \log 1\cdot 5}{\log 20\cdot 5 - \log 9\cdot 0} = \frac{0\cdot 322 - 0\cdot 176}{1\cdot 311 - 0\cdot 954} = \frac{0\cdot 146}{0\cdot 357}$$

or practically 2/5. Other combinations of the observations give a result in close agreement, and we do not consider it necessary to give further detailed calculations of the value of n, which can easily be verified by the reader by reference to the figures.

The interpolation formula

$$0{\cdot}074 + 0{\cdot}3\,(4n+1)/(4n+2)$$

for the nodal position, with $n = 2/5$, becomes

$$0{\cdot}074 + (0{\cdot}3 \times 13/18) = 0{\cdot}291.$$

This calculation is also typical, and will not be given for each individual case, except in its final result.

The ten specimens are shown in figs. 5–14, which are reproduced to scale. These should be compared with fig. 4, which has just reached the critical stage, but in which no whorls have yet appeared.

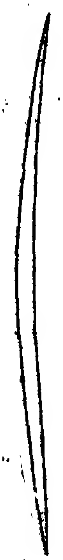


Fig. 4.

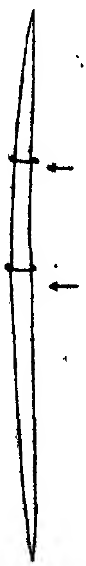


Fig. 5.

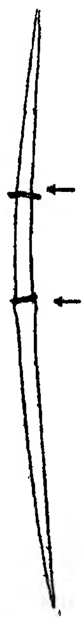


Fig. 6.


Fig. 7.

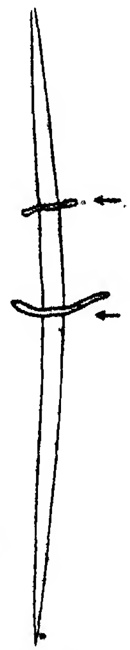


Fig. 8.

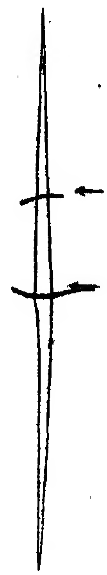


Fig. 9.

The details of these cases are as follows:—

Fig. 5.—Calculations for this spicule are given above. Its length in the drawing is 74·0 mm. and its thickest part is exactly in the middle. The whorls are very near the critical stage (*i.e.*, their first appearance), and the median one is slightly displaced from the centre towards the other, *i.e.*, it

does not quite coincide with the thickest part of the spicule. The distance of this other whorl (the subsidiary whorl) from the end is 20·5 mm., so that the observed ratio of distance to length is

$$20{\cdot}5/74 = 0{\cdot}277$$

against the theoretical value 0·291. The error in the ratio is less than 5 per cent. The theoretical distance of the whorl from the end is 21·5 mm., and the discrepancy is only 1 mm. The displacement is in the same direction as that of the median whorl, apparently a usual phenomenon when the latter is displaced, even though only slightly.

Fig. 6.—This spicule is also very near the critical stage. The lengths on the two sides of the primary central thickening (which is well marked) are 40·5 and 41 mm., the latter being on the side on which the subsidiary whorl is situated The median whorl is nearly 1 mm. from the primary central thickening, but almost at the geometrical centre, and the subsidiary whorl is 26·5 mm. from the end. The ratio of distance to length is

$$26{\cdot}5/81{\cdot}5 = 0{\cdot}325$$

as determined by observation. The spicule is very nearly conical. Typical breadths are 2·1 mm. and 1 mm., at 27·0 and 10·0 mm. respectively from the end. The value of n calculated from these is 4/5, and the interpolation formula yields the ratio 0·314, differing from the observed value by less than 4 per cent. The theoretical distance of the subsidiary whorl from the end is therefore $0{\cdot}314 \times 81{\cdot}5 = 25{\cdot}5$ mm., so that the discrepancy between theory and observation is 1 mm., and as the spicule is slightly curved, so that the theoretical value is really higher, the result may be even more accurate.

Fig. 7.—In this case the median whorl practically coincides with the primary central thickening. The whorls are relatively larger, indicating a slightly more advanced stage of development.

The length of this specimen is again 81·5 mm., and the subsidiary whorl is at 27·5 mm. from the end, giving a ratio 0·337. Typical breadths are 0·5, 1·8, 2·5 mm., at distances 7·0, 27·0, 38·0 mm. from the end respectively, the resulting value of n being 0·937, so that the spicule is almost precisely conical. Calculation gives the theoretical ratio 0·322, again differing from the observed value by less than 4 per cent., while the theoretical distance of the subsidiary whorl from the end is 26·3 mm., giving an error of only 1·2 mm. in the actual distance. This would probably be eliminated if correction were made for curvature.

Fig. 8.—The median whorl and the organic centre of the spicule again coincide very closely, though one end of the spicule has apparently grown a little more than the other, the semi-lengths, measured from the thickest part,

being 44 and 42 mm. The subsidiary whorl is at 28·0 from the end, giving a ratio 0·325. The value of n is 2/3, giving a theoretical ratio 0·310, which is within 5 per cent. of the observed value. The displacement of the subsidiary whorl from the theoretical position is again only 1 mm., and is probably to be accounted for by the curvature of the spicule.

Fig. 9.—The spicule appears to be absolutely conical, and the median whorl is almost precisely central, coinciding with a marked primary central thickening. The true distance of the subsidiary whorl from the end should be $0{\cdot}324 \times 75{\cdot}5$, where 75·5 is the total length. This becomes 24·4, against the measured value 25·4. The displacement is again 1 mm. towards the centre, as required either by curvature or slight subsequent growth at the ends.

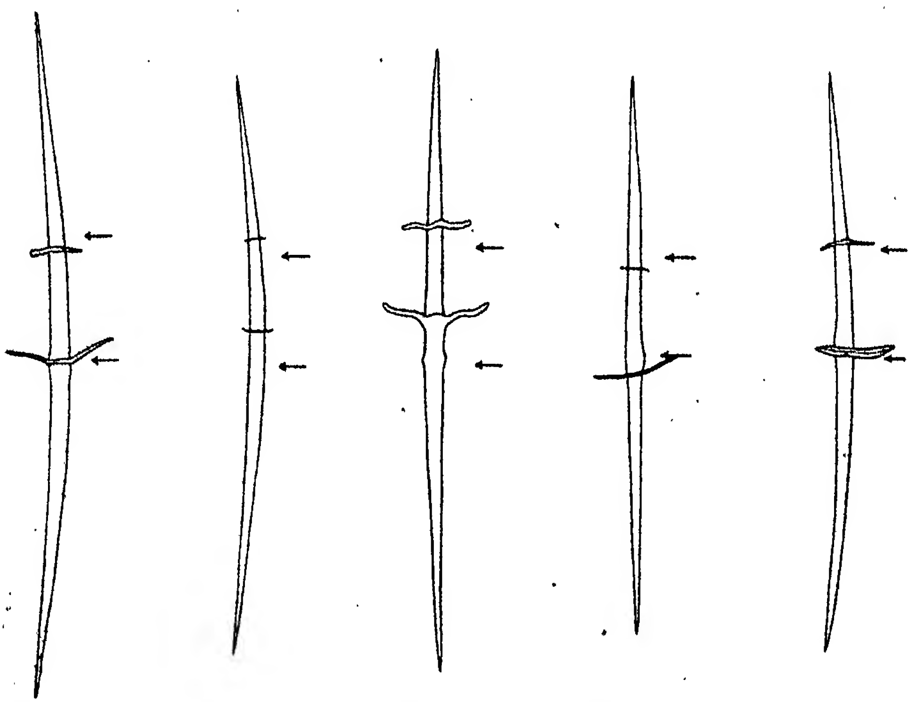


FIG. 10. FIG. 11. FIG. 12. FIG. 13. FIG. 14.

Fig. 10.—The lengths on the two sides of the primary central thickening are 46·0 and 47·0 mm., indicating slightly unequal growth. The median whorl coincides with the primary central thickening. The subsidiary whorl, at 32·0 mm. from the end, gives a ratio 0·344. It is not possible to distinguish the spicule from a conical form, so that the theoretical ratio in the absence of curvature would be 0·324, corresponding to a distance

$0{\cdot}324 \times 93 = 30{\cdot}1$ mm. The displacement is in this case nearly 2 mm. inwards (in accordance with the curvature ?).

Fig. 11.—In this and the following cases there is a very definite displacement of the median whorl from the centre of the spicule, a phenomenon which has already been referred to in connection with fig. 5, and which will be referred to again later on.

The present specimen (fig. 11) is distinctly curved, and apparently very near the critical stage, as indicated by the feeble development of the whorls. A distinct primary central thickening is not recognisable, but the spicule appears thickest at its geometrical centre, from which the median whorl is displaced as much as 4 mm. towards the subsidiary whorl, which is at 22·5 mm. from the end. The whole length is 78·5, and the ratio becomes 0·287. Typical breadths are 1·4 and 2·0 at distances of 22·5 and 35·0 mm. respectively, giving $n = 4/5$ on calculation. This corresponds to a theoretical ratio 0·316, and a theoretical distance 24·8 mm. from the end to the subsidiary whorl. This whorl therefore has a displacement of 2·3 mm. from the theoretical position in the same sense as that of the median whorl. A common factor of displacement, more effective for the median whorl, is thereby suggested.

Fig. 12.—This is a similar specimen to that shown in fig. 11, but shows the primary central thickening clearly and at the geometrical centre. The total length is 84 mm., and the distance from end to subsidiary whorl is 24·0, giving a ratio 0·286. The whorls are relatively large, indicating a stage of development slightly beyond the critical stage.

There is again a displacement to the extent of 5·5 mm. of the median whorl from the geometrical centre towards the subsidiary whorl. The value of n is $4/5$, and the theoretical distance is 26·5 against the observed value 23·5. A displacement of 3 mm. thus occurs in the case of the subsidiary whorl in the same direction as the displacement of 5·5 mm. in that of the median one. The correspondence with fig. 11 is complete even quantitatively, and a common agency effective in causing these displacements must be sought.

Fig. 13.—This spicule is apparently very straight. Its total length is 75·0 mm., and the primary thickening is exactly central. The median whorl is in this case displaced 2·5 mm. on the opposite side to that on which the subsidiary whorl is situated, and we may therefore expect the latter to be more displaced inwards than usual, in order to correspond. This phenomenon actually occurs, for its distance from the end is 26·0 mm., while the theoretical value is 24·3 ($n = 1$, as the form is practically conical). The extra displacement, as in figs. 11 and 12, is roughly half that of the median whorl, although now the displacements are in the opposite direction.

Fig. 14.—The spicule shown in this figure is practically a double cone, of length 77·5 mm. The median whorl is at a distance of 40 mm. from the end more remote from the subsidiary whorl, instead of the theoretical distance $\frac{1}{2}$ (77·5), or 38·75 mm. It is therefore displaced towards the subsidiary whorl to the extent of 1·25 mm. The distance of the subsidiary whorl from the nearer end should be $0·324 \times 77·5$ mm., or 24·0 mm., whereas actual measurement gives a distance of 23·0 mm. The displacement is therefore 1 mm. in the same direction as that of the median whorl.

In attempting to estimate the value of the coincidence between the actual positions of the whorls on the spicule as determined by observation, and the theoretical positions of the nodes as determined by calculation, several possible sources of error have to be considered. In the first place, we have to make allowance for inaccuracies in the drawings which form the basis of the measurements. Such inaccuracies may be expected to arise, partly from the difficulty of making an exact tracing of a very fine line by aid of the camera lucida and partly from the fact that the spicule does not lie absolutely horizontal, one end usually being at a slightly higher level than the other.

We have to remember also that a zone of comparative rest, in which there is but little variation in the amplitude of vibration, extends for some distance on either side of each node, and that the formative cells can hardly be expected to be possessed of the power of determining the exact position of least movement with absolute accuracy. Evidence that they exhibit variations in this respect is suggested by those cases in which the median whorl is shifted a little to one side or other of the central point of the shaft. In such cases, however, the subsidiary whorl also tends to be shifted in the same direction, as though the two rings of formative cells which, on the analogy of *Latrunculia bocagei*, may be assumed to exist, were perhaps linked together, a state of things which, supposing that the cells in question form a sheath around the spicule (as in *Latrunculia*), is very likely to occur. It seems not impossible that alterations of surface tension may be responsible for the dislocation of such a sheath as a whole.

On the whole, it is somewhat surprising to find how close the agreement between the observed and calculated positions of the whorls really is. In order to demonstrate this agreement as clearly as possible, we have indicated, by means of arrows at the side of each spicule, in figs. 5–14, the calculated positions of the nodes for that particular case.

It might be argued that the possible sources of discrepancy are so great as to render mathematical treatment of the subject valueless. That this is not the case, however, is shown by the fact that there is a demonstrable correlation between the shape of the spicule and the position of the whorls,

the tapering form having had its expected effect in shifting the subsidiary whorl towards the centre.

The general tendency of the subsidiary whorl is to be displaced rather farther towards the centre of the spicule than is required by theory. No correction has been made, however, for the curvature of the spicule, which must in itself produce such a tendency, and of a magnitude in general agreement with the observed discrepancies. This curvature cannot be accurately determined, because the apparent curvature must depend upon the position in which the spicule happens to be lying.

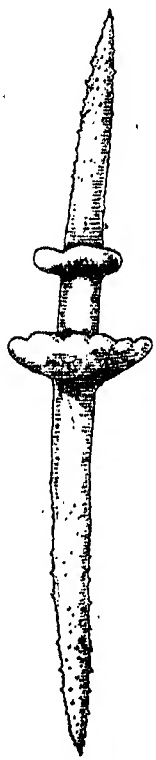


Fig. 15.

It must always be borne in mind, also, that the slight increase in total length of the spicule which takes place after the critical stage has been passed must tend to make the position of the subsidiary whorl appear to approach the centre. Owing to the continued deposition of silica upon the shaft, which results in increase of both length and thickness, the adult spicule is useless for purposes of accurate mathematical investigation of the nodal points. Nevertheless, it is easy to see that, due allowance being made for the disturbing factors, the position of the whorls in the adult spicule is approximately that required by theory. There is surprisingly little variation in this respect, and although we have seen hundreds, or perhaps thousands, of adult individuals, we have never noticed a case which is inconsistent with the vibratory theory. In view of the relative scarcity of individuals at or near the critical stage, the evidence afforded by the examination of large numbers of adults, though it is impossible to carry out that examination with mathematical exactness, acquires considerable importance.

So far as we are aware, no case has as yet been satisfactorily established, at any rate previously to the observations on *Latrunculia* above referred to, in which the occurrence of vibrations during development influences the final form of any part of an organism.* We have here a new factor in

* Since this paper was written we have found a reference, in Prof. D'Arcy Thompson's recently published work on 'Growth and Form,' p. 323, to a suggestion by FitzGerald, that the patterns on the frustules of diatoms might be due to electro-magnetic vibrations. We are not aware, however, that this suggestion has ever been supported by satisfactory evidence. Prof. Thompson himself makes (p. 475) a similar suggestion with regard to the skeleton of certain radiolarians, but without any attempt at mathematical analysis, which, indeed, would be extremely difficult in such cases.

ontogeny, and it will be of great interest in the future to see whether this factor has played any part in the evolution of organisms outside the limits of the Phylum Porifera. We do not propose to speculate upon this subject, but it may be well worth while for botanists and zoologists to keep a look out for possibilities in this direction. No doubt the sponges are especially likely to exhibit cases in which vibrations play an important part, because the water-currents which flow through their canal system with considerable strength must tend to set up such vibrations in any elastic bodies of suitable shape embedded in the almost liquid mesoglœa. But even amongst the sponges, the demonstrable cases, so far as is yet known, are confined to members of the single sub-family Spirastrellinæ, belonging either to the genus *Latrunculia* or to a closely related genus. It is by no means impossible, however, that the form of certain other sponge-spicules may have been influenced by vibrations during development, though we are inclined to think that in most cases the action of surface tension is much more likely to afford a profitable field of investigation. In the case of the oxydiscorhabd, however, it does not seem possible to account for the position of the whorls except by the vibratory theory, and it seems highly probable that the same is true of the *Latrunculia* discorhabd, although the latter has not been submitted to exact mathematical analysis.

The Lateral Vibrations of Bars of Variable Section.

By J. W. Nicholson, M.A., D.Sc., F.R.S.

[This paper, arising out of the foregoing, will appear in Series A of the 'Proceedings.']

OBITUARY NOTICES

OF

FELLOWS DECEASED.

CONTENTS.

Sir WILLIAM GOWERS, 1845-1915.

Sir William Gowers, whose death occurred on May 4th, 1915, was a distinguished member of the medical profession, and in particular a neuropathologist of world-wide reputation.

He was born in London on March 20, 1845, so that at the time of his death he had completed the allotted span of three score years and ten. He was educated at Christ Church School, Oxford, and received his medical education at University College, London, where he was a favourite pupil of Sir William Jenner, to whom in his early professional career he acted as private secretary.

Sir William Gowers became a member of the Royal College of Surgeons in 1867, and took his degree of M.B., London, with First Class Honours in Medicine, in 1869, and M.D., with Gold Medal, in 1870. He was elected a Fellow of the Royal College of Physicians in 1879. His first honorary appointment was that of Assistant Physician to the National Hospital for the Paralysed and Epileptic, with which institution he became specially identified, and which he made his principal field of research and teaching. He was also appointed Assistant Physician and ultimately full Physician and Professor of Clinical Medicine in University College Hospital.

Apart from his neurological work he contributed several articles on diseases of the heart and blood-glandular organs to Reynolds' 'System of Medicine.' In 1878 he invented a hæmoglobinometer, or instrument for estimating the percentage of hæmoglobin in the blood. This was based on the comparison of a tint of an accurately graduated solution of the blood with a standard solution of picrocarminate of ammonia corresponding to a solution of hæmoglobin of 1 in 100. The degree of dilution required to obtain the same tint represents the percentage of hæmoglobin to that of normal blood. This method has been generally superseded by Haldane's modification, in which the standard solution is a 1-per-cent. solution of hæmoglobin saturated with carbon monoxide. The blood to be tested is similarly treated by passing a stream of coal gas through it.

Gowers also devised an improvement of Hayem's hæmocytometer, or instrument for counting the blood corpuscles, by ruling the micrometric squares on the bottom of the cell instead of on the eyepiece of the microscope. This method, though giving fairly accurate results, has now been generally superseded by the more simple Thoma-Zeiss instrument.

In 1897 he published a work of 'Medical Ophthalmology' which, with its beautiful illustrations, all drawn by his own hand, did much to popularise the routine use of the ophthalmoscope in medical diagnosis, so strongly advocated by his eminent colleague Dr. Hughlings Jackson more than ten years before.

Sir William Gowers' first important work in neuropathology was his

'Diagnosis of Diseases of the Spinal Cord,' published in 1880. This, though only a small octavo of 80 pages, was a very lucid exposition of the anatomy and functions of the spinal cord, and of the methods of diagnosis of spinal lesions. In this work he was the first to describe a tract of ascending degeneration in the antero-lateral column consequent on a crush of the lower part of the cord by a fracture of the spine. It is now known generally as Gowers' tract, or ascending antero-lateral fasciculus. There is still some uncertainty as to its exact origin and ultimate destination.

In 1881 he wrote a book on 'Epilepsy' and other convulsive disorders—the substance of his Gulstonian Lectures at the College of Physicians—founded on his own observations of several thousand cases, chiefly at the National Hospital. But his great work, which embraced all his separate contributions to neuropathology, was his 'Manual of Diseases of the Nervous System,' in two volumes. The first was published in 1886, the second two years later. This at once established Gowers' reputation throughout the medical world as a neuropathologist of the first rank. It was speedily translated into the more important modern languages. Three editions of the first volume have been published, and two of the second. The third edition of the second volume was never completed.

Gowers' manual, admirable as a text-book—concise, lucid and well arranged—was not, like many text-books, a mere compilation, but revealed on almost every page the fruits of independent clinical and pathological investigation extending over many years. The work was admirably illustrated, many of the figures having been drawn by his own hand. It is not too much to say that no better manual of nervous diseases has ever been written in this or any other language. It still maintains its position, though many other similar treatises have been published, both at home and abroad.

Sir William Gowers was an excellent teacher, and his clinics at the National Hospital were largely attended by students from all parts of the world. He had the reputation of being somewhat too dogmatic, but this, perhaps, to the majority, made his teaching all the more impressive.

Many academic and other honours were conferred on him. He was Fellow of University College, Hon. M.D. of Dublin, LL.D. of Edinburgh, Hon. Fellow of the Royal College of Physicians of Ireland, Hon. Member of the American Neurological Association, the Netherlands Society of Psychiatry and Neurology, the Russian Society of Medicine, the Royal Society of Medicine of Upsala, and of the Society of Internal Medicine of Vienna.

He was elected Fellow of the Royal Society in 1887, and received the honour of Knighthood in 1897.

Sir William Gowers had a large practice as a consultant, but failing health led to his retirement several years before his death. In person he was of medium height, spare figure and nervous temperament. He was not a clubable man, and few of his friends and colleagues ever got to know him intimately. He seldom attended learned societies, and then only when he

was invited to deliver some address. He was a forcible and sententious speaker.

He was a skilful draughtsman and an etcher of considerable artistic merit. He was also an expert shorthand writer, and was the founder of the Society of Medical Phonographers, to the pages of whose journal he was a frequent contributor. He had no active recreations, but in his holidays spent a good deal of his time in etching, and in the study of mosses, of which he had an extensive knowledge.

Sir William Gowers married the daughter of Frederick Baines, of Leeds, in 1875. She predeceased him in 1913. Two sons and two daughters survive their parents. The death of Sir William Gowers removes from the roll of the Royal Society one of the most eminent of the physicians who have attained this honour.

D. F.

RICHARD LYDEKKER (1849—1915).

Richard Lydekker was born on July 25, 1849, at 45, Tavistock Square, London, the eldest son of Mr. Gerard Wolfe Lydekker, barrister-at-law, who shortly after his son's birth purchased Harpenden Lodge, Harpenden, where Richard lived practically for his whole life, and where he died on April 16, 1915. The family was of Dutch extraction, but had been domiciled in this country and connected with Hertfordshire for several generations, Lydekker's father having been a magistrate in that county, and his grandfather, after being Governor of Martinique, having lived and died at St. Albans.

Lydekker entered Trinity College, Cambridge, in 1867, gained second place in the First Class Natural Science Tripos, and took his B.A. in 1872. Two years afterwards he was appointed to the Geological Survey of India, in whose service he remained until the death of his father in 1881. While in India he explored very systematically the mountain ranges of Kashmir, and gained a knowledge of the geography of that complicated region which was afterwards of much use to him in his zoological work, and when the Survey collections in Calcutta were transferred to the newly built Indian Museum, his keen biological bent found scope in the arrangement and description of the series of tertiary vertebrate fossils, to which large additions had recently come from the Punjab. A permanent record of his work at that time remains in his contributions on the Siwalik fauna in the 'Palæontologia Indica.'

He returned home to England in 1882 after his succession to the family home at Harpenden, where he took up his father's magisterial work in the

county, and then commenced his long connection with the Natural History Museum, first as a palæontologist, and later as a worker on recent Mammalia.

Under the auspices of Dr. Henry Woodward, he undertook the preparation of a Catalogue of the Fossil Vertebrates in the British Museum Collection, a publication consisting of four volumes of Mammals (1885–87), two of Reptiles (1888–90), and one of Birds (1891). While doing this he wrote a large number of palæontological papers dealing with questions arising out of his study of the collections.

In preparing this work Lydekker was more and more brought into contact with the staff and collections on the Zoological side of the Museum, and when in 1891 Sir William Flower needed a colleague to work up his Encyclopædia articles on Mammals into a separate volume, he found Lydekker able and willing to help him to do so. The resulting volume, 'An Introduction to the Study of Mammals,' was perhaps the most valuable work bearing Lydekker's name, and formed for many years the standard work on systematic mammalogy. Nor has it yet been superseded by any other. In a similar way, with Prof. H. A. Nicholson, he prepared the third edition of the latter's 'Manual of Palæontology,' one of the best text-books on the subject.

In 1893 Lydekker accepted an offer by Dr. F. P. Moreno, of the La Plata Museum, to go out and examine some of the wonderful series of Mammalian fossils which had been described by Argentine zoologists, especially by the late Señor F. Ameghino, a visit which was of very great interest to him, and largely increased his knowledge of South American fossils. Great discussion had arisen as to the age of the beds in which the fossils were obtained, notably the so-called "Pyrotherium beds," and he adopted the view, now generally accepted, that they were considerably more recent than had been claimed by Ameghino. But in the somewhat embittered polemics between Moreno and Ameghino he declined to take any definite part.

In January, 1896, Lydekker was engaged to reorganise the Mammalian Exhibition Galleries of the Natural History Museum, which had been up till then in the nominal care of the Assistant in charge of the Mammalia. The latter's time, however, was necessarily so much given to the vast and rapidly increasing study collections that little attention could be paid to the arrangement of the Exhibition, in which Sir William Flower took himself such personal interest.

This arrangement, which was in force to the end of his life, was a most admirable one, releasing the Museum Assistant from a work which he had not time to do properly, and giving Lydekker an opening of which he took immediate and continuous advantage. As a result, the whole Exhibition series of Mammalia has been completely remodelled and enormously increased in extent and interest, and the specimens better mounted and better labelled. He also similarly rearranged the galleries devoted to Reptilia and Mammals in the British Vertebrate series. The official guides to these galleries were mostly written and revised by him.

In connection with his Exhibition work, Lydekker became more and more interested in large Mammals, and, as a consequence, in the sporting side of Natural History, writing constantly for the 'Field' and similar papers, and gradually becoming the chief referee for all questions connected with the technical side of Sporting Zoology. And when recently the Trustees decided to publish a Catalogue of Ungulates on the same lines as the other technical catalogues, a work which for various reasons was of quite unusual difficulty, it was entrusted to Lydekker as the chief authority on the subject. Commencing it in 1913, and working with his usual celerity, he had practically finished it by the time his fatal illness ended, working on his sick bed at the proofs of the fourth and the MS. of the fifth and last volume.

Side by side with the Exhibition and Catalogue work, Lydekker prepared for 27 years (1887–1913) the Mammal part of the 'Zoological Record,' for which his genius for hard work and his wide knowledge of Mammalia made him peculiarly suitable. He also took very great interest in Geographical Zoology, his 'Geographical History of Mammals' (1896) being one of the best books on the subject.

The most striking characteristic of Lydekker was his ceaseless activity in publishing work on the subjects he was interested in. Technical or popular, an absolutely unbroken stream of writing came from his pen from the early nineties to the date of his death, and it is probable that no scientific writer has ever produced so much in the time as he did during the last 20 years of his life. Though much of it made no pretension to be of permanent value, all shows how intensely interested he was in every branch of his subject, and how wide his sympathies were with other workers. Personally, the writer of this notice has found him again and again the one zoologist who could give intelligent interest on technical points to which few modern mammalogists trouble to pay any attention. On all such technical matters—as, for example, the wonderful dentition of the Manatee, which he worked out in conjunction with the writer, the ancestry of the Sirenians (see 'Proc. Zool. Soc.,' 1892), and other similar subjects—his interest was intense and helpful, even if the exigencies of his life led him to devote most of his publishing energies to the more sporting and popular side of Zoology. In official matters he was ever helpful, and ready to do anything he was asked.

His rapidity of work was phenomenal, but, unfortunately, in conjunction with a somewhat illegible handwriting, this brought its penalty in a rather undue proportion of misprints and *lapsus calami*, such as a slower worker might have avoided. On this account some of his writings hardly do justice to his really great knowledge of his subject.

At home at Harpenden, besides his unceasing writing work, his occupations and amusements were chiefly stamp collecting, walking, gardening, and carpentry. In youth he had been fond of shooting, but was never bitten with the craze for games of any sort, though he took a sympathetic interest in the sporting careers of those who were.

In person he was tall, over 6 feet in height, handsome and well

proportioned, with light hair and blue eyes, his foreign ancestry coming out in a somewhat Scandinavian or North Dutch appearance. In politics he was a keen Unionist and Imperialist, but, beyond occasionally presiding at the Harpenden meetings of the party, he was not a demonstrative politician.

He joined the Zoological Society in 1880, and served on the Council in 1898–1900. He was elected a Fellow of the Royal Society in 1894.

He married in 1882 Lucy Marianne, elder daughter of the late Canon O. W. Davys, Rector of Wheathampstead, and had two sons—of whom the younger has fallen on behalf of his country and the elder is now serving—and three daughters.

O. T.

SIR JOHN MURRAY, K.C.B., 1841–1914.*

Sir John Murray was born on March 3, 1841, at Coburg, Ontario. He came of one of those Scottish families that have done so much for Canada, and, indeed, throughout his life no one would have mistaken him for anything but a Scot. His father, Robert Murray, an accountant, had left Scotland seven years before and settled in Upper Canada, where during the troublous times of the Mackenzie Rebellion he took an active part in Canadian politics. John was for a time at the Public School of London, Ontario, and later at Victoria College, Coburg. When he was seventeen years old he left Canada and, as he has himself reminded us, he then for the first time saw the sea whose problems he was destined to make his own. When he left that early home, he says, "to find another amongst my relatives in Scotland, I had not yet seen the ocean. The voyage across the Atlantic made a great impression on me, so different was the salt, rolling sea from the great fresh-water lakes with which I had up to that time been familiar, and I was fascinated by the operations of the officers on the bridge when taking the altitude of the sun at each mid-day." On witnessing the rise and fall of the tide for the first time on the West Coast of Scotland, the impression was still more profound.

John Murray found a new home amongst his Scottish relatives, one of whom was John Macfarlane, his maternal grandfather, at Coneyhill,

* In writing this short memoir of my friend I have been greatly helped by Mr. Laurence Pullar, of Bridge of Allan and Bridge of Earn, by Dr. Hugh Robert Mill, by Dr. J. Sutherland Black and by Mr. James Chumley, who for many years was Sir John Murray's chief assistant.

Stirlingshire. He helped his grandfather in purchasing and collecting specimens for a museum, the remnants of which are still exhibited in the Macfarlane Institute at Bridge of Allan, many of the labels being in Murray's handwriting. Whilst living with his Scottish relatives he attended the High School, Stirling, and here he showed great interest in science. He used to pay especial attention to the teaching of Mr. Duncan Macdougall, from whom he learnt the principles of the sextant and how to construct an electric lamp and a battery of 80 Bunsen cells.

Murray remained for a long time at School and College. In fact, as he himself records, he came to be known as a "chronic student" at the University of Edinburgh. One thing he would not do, he would not go in for examinations. He learnt what he wanted to learn, and the mere learning was to him its own reward.

At the University, although in the main he followed the Science course, he was not infrequently to be seen in the lecture rooms of the literary professors and from time to time in those of the theological professors. Amongst his student friends more than one have made a mark on the theological thought of the last half of the nineteenth century. Occasionally he even listened to Law. His Zoology and Anatomy he studied under Goodsir and Turner, the present Principal, whilst he worked at Chemistry with Playfair and Crum Brown, and at Natural History with Allman. But undoubtedly the teacher who made most mark upon his mind was Prof. Tait, in whose laboratory he worked for several terms under William Thomson, (afterwards Lord Kelvin), Clerk Maxwell, and with his life-long friend, Robertson Smith, who at that time was demonstrator to Tait and was writing more than one mathematical paper of note. Later Robertson Smith became a distinguished Semitic scholar, one of the editors of the 9th edition of the 'Encyclopædia Britannica," and after a theological controversy with the Free Church of Scotland a Professor of Arabic in the University of Cambridge, and, finally, University Librarian.

Tait was then, perhaps, at the height of his reputation and many students of various sorts were attracted to his laboratory; Sir John Jackson and Mr. Meik, the celebrated engineers, were amongst the young physicists, and curiously enough Robert Louis Stevenson was another. The last named, however, had no interest in science and used to beguile his demonstrator, Robertson Smith, into theological disputes, so dear to all true Scots.

Murray was always a great individualist, and he worked at what interested him with no eye to examinations or degrees, and although in later life he must have been surfeited with honorary degrees, as a student he passed by the examinations and the consequent degrees and never graduated.

In the year 1868, in a spirit of adventure and on the strength of having attended medical classes in Edinburgh, Murray accepted the post of surgeon on the whaler "Jan Mayen." He left Peterhead in February, and was away seven months. He saw a good deal of the Arctic regions, explored part of Spitzbergen, and landed at least once on Jan Mayen. During his

absence his grandfather died, and Murray arrived home two days after the funeral to find that—unlike Loudon Dodd—he had been cut out of his grandfather's will with less than the proverbial shilling. It was the experience he gained on this Arctic voyage and during his subsequent work on the West Coast of Scotland in the years 1869 and 1870 which qualified him for his next post.

Murray's great chance in life came when the Government decided, on the recommendation of the Royal Society, to equip a surveying ship, the "Challenger," for scientific research and to send her round the world. "The 'Challenger' was a spar-decked corvette of 2306 tons, with auxiliary steam to 1234 horse-power," and was well adapted for the scientific purposes to which she was devoted for four years. The scientific staff was under the direction of Prof. (afterward Sir) Wyville Thomson, of Edinburgh University, and at first John Murray was not included on it; but at the last moment, owing to the failure of one who had been chosen, on the earnest advice of Prof. Tait, John Murray was selected for the vacant post. Tait especially dwelt upon the fact of Murray's resourcefulness and readiness, and considered he would be a very useful man to have at hand in case of any difficulties with natives or other possible sources of trouble. It was characteristic of Murray to embark on such an enterprise at a moment's notice, when there was almost no time to get together his scientific or personal "kit."

But the science of the depths of the sea and the science of oceanography were in those times inchoate. The first great expedition to investigate the physical, the chemical, the geological, and the biological conditions of the great ocean basins was sent out in 1872 by the Government of this country, then under Mr. Gladstone, and in that year H.M.S. "Challenger" left England with a staff of scientific observers to traverse the salt waters of the globe. From that date until the present time no such complete and organised a staff of scientific observers, helped in every way by the naval officers (for it was an Admiralty Expedition), has left any country for so prolonged and exhaustive an investigation into the economics of the ocean. The "Challenger" Expedition set a standard—in fact it practically established a new science, a science of which Sir John Murray was, in a way, the arch-priest.

The "Challenger" Expedition had predecessors, though on a much smaller scale. Maury had done a great deal in the way of the study of the ocean, especially in so far as concerned its depth and the ocean currents. Dr. Wallich on H.M.S. "Bulldog," surveying the route for the proposed Transatlantic cable, added much to our knowledge, and there were others. The immediate precursors of the expedition of the "Challenger" were a series of voyages made by the "Porcupine" and "Lightning" under the scientific guidance of Dr. W. B. Carpenter, Mr. Gwyn Jeffries, and Prof. Wyville Thomson. Dr. W. B. Carpenter took an immense interest in the question of deep-sea temperatures, and read a number of papers to the Royal Society dealing with all existing data accumulated down to 1870, and

he was one of the leading spirits in stirring up that Society to urge the Admiralty to undertake the "Challenger" Expedition. At the Admiralty they were aided by the then hydrographer, Admiral G. H. Richards, who was extremely sympathetic with the work.

As the introduction to the narrative of 'The Cruise of the 'Challenger'' recites: "The vast ocean lay scientifically unexplored. All the efforts of the previous decade had been directed to the strips of water round the coast, and to enclosed or partially enclosed seas. Great things had certainly been done there, but certainly far greater things remained to be done beyond. This consideration led to the conception of the idea of a great exploring expedition which should circumnavigate the globe, and, if possible, find out the conditions of life at the surface of the sea, at the intermediate depths, and also at the profound abysses of the ocean. Sir John Murray's main interest in the expedition was at first physical and geological rather than biological, though he soon acquired a real knowledge of animals, at any rate in so far as they affected the problems which appealed more nearly to him." He was an adept at criticising machines and instruments which plumb the secrets of the deep, and as soon as the results of his researches on the bottom of the deep sea had appeared he was recognised at once, and as long as he lived, as *the* authority on the deposits covering the floor of the ocean.

Sir John was no specialist. He had ever the widest point of view of the chemistry, the physics, the geology, and the biology of the ocean, and to him these varying sciences always had their full value in the problem which he had made his own. He was constantly devising new sounding apparatus for bringing up samples of the sea bottom, thermometers for testing the bottom temperature, instruments for registering the pressure at great depths, and other implements which have made our knowledge of the depths of the sea accurate and even minute.

The ship sailed from England, quite at the end of 1872, with John Murray on board as Naturalist at a salary of £200 per annum. From the time of its departure Murray gave especial attention to the various oozes and other deposits which compose the floor of the ocean, and at an early period he came to the conclusion that Bailey, Johannes Müller, Count Pourtales, Krohn, Max Schultze and Ernst Haeckel were right when they attributed certain of the minute shells at the bottom of the ocean to organisms which live nearer the surface. Murray correlated the contents of the surface tow-net with the results of soundings and found a close relation to exist between the surface fauna of any locality and the deposit which lies beneath it. Amongst other organisms he paid much attention to the curious coccospheres and rhabdospheres, as Murray now for the first time called them. He devised an ingenious method of abstracting these extremely minute organisms from the sea-water by stretching pieces of fine thread through a bucket of salt water and allowing it to stand for the night. The examination of the threads next morning showed these organisms entangled among the strands. Another unfailing source of supply of these curious, and still imperfectly understood,

organisms was the stomachs of the Salps, whose pharynx, fine as its walls are, allowed these organisms to pass through its narrow-meshed sieve.

'The 'Challenger' Report on Deep-Sea Deposits' by Murray and Renard was published in 1891. It was the first attempt to deal with marine deposits as a whole, and became at once the standard book on this subject, a position it occupies to the present day. It was in every sense of the word "epoch-making." The amount of research work entailed in the preparation of this monograph was stupendous; the detailed microscopic study and chemical examination of thousands of deposit-samples from all parts of the world and from all depths, and of the various constituents contained therein, involved the expenditure of much time and labour.

The terms applied to the various types of deposits, with the exception of "*Globigerina* ooze," already in use before the time of the "Challenger" Expedition, were devised by Murray on board the "Challenger," some of them being subsequently more or less modified in collaboration with Renard. The nomenclature and classification finally adopted by them have stood the test of time. Notwithstanding the numerous contributions to the subject published in the interval, and the many attempts to improve upon either the divisions, the terms, or the methods originally employed, the "Challenger" Report remains the model and standard upon which all studies of deep-sea deposits are based, and it appears to satisfy all the demands made upon it. This is conclusive evidence of the abundant foresight, care, and scientific precision brought to bear upon the study of the "Challenger" material and of material collected by other ships up to the time of publication.

Murray came to be recognised as *the* authority on all matters relating to the floor of the ocean. His reputation became world-wide, and his advice was solicited on all hands in connection with the fitting out of expeditions and with the scope of deep-sea researches of various kinds. Needless to say his extensive knowledge and practical experience were freely placed at the service of scientists, and many further additions to our knowledge of the sea and its laws are due to his initiative.

The bottom samples collected by nearly all the surveying ships, cable ships, and oceanographic expeditions of all nations, found their way to the "Challenger" Office in Edinburgh for examination and report, and Murray was thus enabled to bring together a magnificent collection of marine deposits, a collection which is unique in the world.

One may quote here an appreciation of his work given by the well known Arctic explorer, General Greely. Writing two years ago in the 'National Geographic Magazine,' of Washington, U.S.A., General Greely says: "Nearly 40 years since, a distinguished scientist, born on the continent of North America, Sir John Murray of 'Challenger' Expedition and fame, and one of the eight honorary members of the National Geographic Society, considered the mooted extent of South Polar lands and finally outlined their logical continental form as the continent of Antarctica—a fitting and largely accepted name. This great feat of constructive geography depended on a few

score handfuls of oceanic ooze from the South Polar seas and scanty bits of rocks from scattered lands. Whatever doubts remained as to the accuracy of Murray's deductions have disappeared since the cumulative discoveries of Amundsen, Borchgrevink, Bruce, Drygalski, Gerlache, Larsen, Nordenskiöld, Scott and Shackleton."

Secondly, Sir John did much to throw light upon the origin of coral reefs. At the time of the "Challenger" Expedition Darwin's theory of subsidence held the field, but Murray, who proved all things and held fast only to that which he conceived to be true, found occasion to doubt its universal application. The boring at Funafuti, an island which was especially selected alike by the opponents and adherents of Darwin and Murray respectively as a typical place for investigation, clearly proved that Darwin was right in some places; there is room enough in the world for some coral islands to have been formed by sinking and others by the rising of the earth's crust. Darwin himself always admitted, after the publication of Semper's memoir, that his subsidence theory was not of universal application.

At the time of Murray's return from the "Challenger" Expedition, Sir Archibald Geikie, O.M., was Professor of Geology at the University of Edinburgh, and Murray then attended his lectures. Sir Archibald helped him in the preparation of the geological section of the "Challenger" Reports, and Murray took an active part in the many excursions which are ever the delight of the geological student. Sir Archibald has kindly written the following lines:—

> "During the preparation of the geological parts of the 'Challenger' Reports we had long talks over the problems suggested by the observations made on the voyage. He was always an original and suggestive thinker in connection with these problems. Nowhere are his originality and acuteness more conspicuous than in his discussion of the origin of coral reefs. Up to this time, Darwin's explanation held the field, though a few observers had challenged its universal application. But when Murray published his views, in which he combated the proofs of vast oceanic subsidence and held that all the types of coral reef could be accounted for without subsidence and even with local elevation, he effected one of the most striking revolutions in geological theory which have taken place in our time. When Alexander Agassiz took up the question and made a prolonged series of expeditions over the coral regions of the oceans he brought a vast mass of fresh material in support of Murray's opinions. While I think it quite possible that here and there Darwin's explanation may be found to hold, I feel tolerably certain, after Agassiz's ample succession of exploration, that Murray is right for the general origin of coral reefs over the globe.
>
> "Then Murray's laborious researches into the nature and distribution of the materials that are accumulating on the ocean floor and his classification of them broke entirely new ground in the Dynamical section of

Geology. Many a long discussion he, Renard, and I had on this subject, and it was a delightful experience to watch how, bit by bit, out of the vast mass of materials collected by the 'Challenger,' there emerged the clear and impressive generalisations which were embodied in the 'Deep Sea Deposits.' Murray and Renard, by this remarkable volume, rendered a noble service to Oceanography and to our knowledge of the geological process now in action in the oceans.

"Murray's later work on the Scottish lakes is another example of his originality and thoroughness. He not only planned this work with great skill and wide knowledge but, as it proceeded, he threw into the labours of his associates much of his own enthusiasm and devotion."

During the time that Murray was seeing the "Challenger" Reports through the press he was engaged with his friend, the late Mr. Robert Irvine, and others, on a series of chemical investigations upon the secretion of carbonate of lime from sea-water by marine organisms and on the part played in this process by the waste products given off during their nutrition. He also worked at the bacteriology of the deep-sea deposits, developing the work of the Russian oceanographers on the sulphuretted hydrogen bacteria of the Black Sea. The series of papers recording these researches appeared in the 'Proceedings' of the Royal Society of Edinburgh.

The third investigation, referred to by Sir Archibald, on which he embarked in his latter years, was that of the bathymetric survey of the fresh-water lochs of Scotland. The Councils of the Royal Societies of London and of Edinburgh had urged the Government to undertake this survey. The Government did not feel that this enterprise came within the province of the Ordnance Survey Office nor within that of the Hydrographic Department of the Admiralty, but when Murray wanted a thing done, in the long run it generally was done, and he and Mr. Frederick Pullar in 1896 commenced the work and had already published some papers of importance when, by the accidental death of Mr. Frederick Pullar by an ice accident in 1901, the work was interrupted. His father, however, Mr. Laurence Pullar, determined to see the work through, and provided a large part of the funds used for this purpose, and in 1902 a staff of assistants was appointed to resume the survey. For the next four years the surveying work was vigorously carried on, and some 60,000 soundings were recorded from no less than 562 inland lakes. Biological and physical observations were also carried on during the two following years, and the results of this, the most careful survey ever carried out on the inland waters of any country, were published in six handsome volumes in 1910.

One would have thought that three such problems as Deep-Sea Deposits, the Origin of Coral Islands, and the Fresh-Water Lochs of Scotland, would have exhausted the energies of any man, but Sir John seems to have been tireless in his activity. Besides editing the 50 volumes of the "Challenger" Reports, he was the author of the summary of the scientific results of the

expedition in two large volumes. As he records, "The direction of the whole of the work connected with the publication of the scientific results passed unexpectedly into my hands, and I have done my best under the circumstances to place on permanent record a trustworthy account of the labours of this famous expedition. It has been my earnest endeavour to complete the publication in a manner worthy of the naval position and the scientific reputation of this great Empire. Notwithstanding troubles, personal sacrifices and regrets necessarily connected with the work, it has been a pleasure and an honour to have taken part in the explorations and researches which mark the greatest advance of the knowledge of our planet since the celebrated geographical discoveries of the 15th and 16th centuries."

He was never tired of exploring the sea, and in 1880 and 1882 he took part in two expeditions to explore the Faroë Channel in H.M.S. "Knight Errant" and H.M.S. "Triton." He established marine laboratories first of all at Granton on the Firth of Forth, and later on the Clyde at Millport, Cumbrae. Between 1883 and 1894 he was continuously exploring the West coast of Scotland in his small steam yacht "Medusa," which was specially fitted for carrying on oceanographical investigations, and in these he was assisted by Mr. J. T. Cunningham, Dr. H. R. Mill, and many naturalists.

He never spared himself, and when he was approaching his 70th birthday he embarked on the "Michael Sars," a steamer no bigger than an ordinary fishing trawler, with a gross tonnage of 226 and with but 300 h.p. engines, to cross the Atlantic on a scientific expedition, the profoundly important results of which he published in collaboration with Dr. Johan Hjort in the well known book, 'The Depths of the Ocean.' He was very capable of getting on terms with the sailor men, and had a thorough knowledge of the sailor's mode of life and the sailor's point of view, and, it may perhaps be mentioned, of the sailor's vocabulary. Although he became 73 a few days before the tragedy, he seemed, and was, in fact, a much younger man, "good for at least another 10 years," as a leading physician, who knew him well, remarked to me some weeks ago.

He took a great interest in the project for establishing a meteorological observatory on the top of Ben Nevis. He was Secretary of the Committee which raised the necessary funds, and largely through his efforts £5000 was soon collected. He was one of the Directors of the Observatory until, unfortunately, it was closed a few years ago. For several years he was a scientific member of the Scottish Fishery Board, and he represented the British Government at the International Fisheries and Hydrographic Conference in Stockholm in 1899, and he was President of the Geographical Section of the British Association in 1899. The same year he delivered the Lowell Lectures at Boston, U.S.A., and again in 1911 he delivered a second course of Lectures at the Lowell Institute. For many years he ungrudgingly gave his services as one of the Secretaries and Member of the Council and Vice-President of the Royal Society of Edinburgh, and the societies with which he was actively connected are almost as numerous as the honours

which in later days were showered upon him. At the time of his death he was President-elect of the Meteorological Conference to be held in 1914 at Edinburgh, and was actually engaged in making arrangements for a successful meeting the day before his tragic end.

Sir John held strong views on Education. He had little use for the "grand fortifying curriculum" of the Classics, but I shall never forget how indignant he was with me when a few years ago I was unable to produce at almost a moment's notice a tutor for his son, who was to be at once "a first-class classic and a thoroughly trained oceanographer." His son was then reading for the Previous Examination and embarking on a voyage round the world.

Apart from his science, which would have occupied the entire time of most men, Sir John was latterly also interested in commerce. A bit of material included among a collection of deep-sea deposits from the neighbourhood of Christmas Island in the Indian Ocean enabled him to recognise that that remote island contained valuable phosphatic deposits. The island was quite uninhabited, but obviously a source of wealth, and he urged the Government to annex the island. Ultimately they did so, and Sir John obtained a lease of it along with Mr. Ross, of the Cocos Islands. A company was formed to develop its resources, and Mr. C. W. Andrews, of the Geological Department of the British Museum, was granted leave of absence for a year, and in 1897–8 he visited and explored the island, Sir John paying all the expenses and presenting the specimens Mr. Andrews collected to the British Museum. The Trustees in 1900 published the result of the researches in a monograph, which is a most interesting record of the indigenous animals and plants of a lonely oceanic island both before the arrival of civilised man and after.

Sir John himself on several occasions visited Christmas Island, and crossed it from end to end. Valuable deposits of phosphates were found, water was discovered, clearings were made, a railway laid down, waterworks and piers constructed, aerial haulage erected, and houses built. The island now maintains a population of about 1500, composed of Europeans, Colonials, Chinese, Malays, Sikhs, etc., and a flourishing business is being carried on in the export of phosphates. Plantations of rubber, hemp, coconuts, bananas, papaws, cotton, etc., have been established with more or less success. Sir John always looked upon this development as an indirect result of the scientific work of the "Challenger" Expedition, and an excellent argument for such research work being carried on by the Government. He knew that His Majesty's Treasury had received in hard cash within the past 15 years, in the form of rents, royalties, and taxes, a sum greater than the cost to the country of the whole "Challenger" Expedition, and he recalled how, during the time the money was being annually voted for the issue of the "Challenger" publications, many Members of Parliament objected to public money being voted for such a purpose.

To enumerate the various honorary degrees, honorary memberships of learned Societies, medals and decorations of all sorts that Sir John received

would occupy too much space. The more important of them are set out in 'Who's Who,' but he always held that they were conferred on the Expedition rather than upon himself.

In stature Sir John was short, broad shouldered, with a finely poised, distinguished head. His complexion was fair and his blue eyes piercing. His was a personality that could not be overlooked in any company. He was at times brusque, rather domineering, very confident of his own opinion, and he liked to have his own way, and, indeed, he generally got it, but he was most kind and most helpful to his assistants, and he spent his wealth largely in promoting the advance of science. He was singularly straightforward and at times almost blunt, but he did not understand or appreciate the methods of the politician. If he was once your friend he remained your friend. Rather late in life he married in 1889 Isabel, only daughter of the late Mr. Thomas Henderson, of the well known Anchor Line of Glasgow. He was a devoted husband and father, and although he had unconventional ideas about the education of his children he was profoundly attached to them, and was never happy unless he had one or other with him.

Sir John Murray was instantaneously killed in a motor accident near Edinburgh on March 16, 1914. A. E. S.

DAVID DOUGLAS CUNNINGHAM, 1843–1914.

David Douglas Cunningham was born at Prestonpans on September 29, 1843, shortly after his father, the Rev. W. B. Cunningham, minister of the parish since 1833, left his manse and followed Dr. Chalmers into the Free Church of Scotland. His paternal grandfather, Captain Robert Cunningham of the Berwickshire Militia, was himself the grandson of a former minister of Prestonpans, appointed in 1722. Cunningham's mother was the daughter of David Douglas of Reston, Sheriff of Perthshire, afterwards Lord Reston and a senator of the Scottish College of Justice, son of Colonel Robert Douglas of Strathendrie. His maternal grandmother was a granddaughter of Lord Craigie, Lord-Advocate of Scotland in 1745. She and her husband, who were cousins, both bore the same relationship to Adam Smith, author of the 'Wealth of Nations,' a moiety of whose library Cunningham inherited.

Cunningham went to school at the Queen Street Institution in Edinburgh, proceeding thence to the University, where he graduated, with honours in Medicine, in 1867. In April 1868 he gained a commission in the Indian Medical Service and entered Netley.

The interest then taken in the theories advanced in Germany as to the causation of cholera induced the Senate of the Army Medical School to move the Secretaries of State for India and for War to employ on a special enquiry into this disease the two officers who, at the close of the summer session of 1868, should secure the highest places in the Indian and the Army Medical Services respectively. Cunningham, who headed the combined list both on entering and when leaving Netley, was the young Indian officer so chosen; his colleague from the sister service was the late Dr. T. R. Lewis, whose early death in 1886, after his selection but before he could be elected into the Royal Society, was much deplored by his contemporaries.

After a visit to the late Rev. M. J. Berkeley, F.R.S., to study the methods of investigation employed by that eminent mycologist, Cunningham in company with Lewis went to Germany to learn the views and master the technique of Hallier and De Bary, and to work for a time under Pettenkofer at Munich. Returning to England in December the two young officers at once left for India. They reached Calcutta in January 1869 and were attached as special assistants to the department of the Sanitary Commissioner. For ten years thereafter both were continuously engaged in important pathological and hygienic studies. When at their headquarters in Calcutta this work as a rule was done in collaboration; when they were on deputation in the provinces it was undertaken independently. The special cholera enquiry originally entrusted to them began from the date of their leaving Netley and lasted until May 1879, this service being rendered by Cunningham as an officer of the military establishment until December 1874, when he was transferred to the Civil department for additional employment in general enquiries into other special forms of disease. His absences from headquarters during 1869-79 included tours in Madras during the cold season of 1870-71 and in North-west and Central India during the cold season of 1872–73; the hot weather of 1876 he spent in North-west India, that of 1878 in Madras and that of 1879 again in North-west India.

In 1879 administrative considerations induced the Government of India to merge the department of the Sanitary Commissioner in that of the Surgeon-General. This change of organisation brought to a close the long association of Cunningham and Lewis, so fruitful in useful results. In anticipation of the alteration the services of Cunningham were placed by the Home department of the supreme government at the disposal of the Government of Bengal, who had resolved to establish a chair of physiology in their medical college at Calcutta. Cunningham was appointed to this post in June 1879, but as Lewis had been granted leave out of India and as the work of the new chair did not commence until the opening of the winter session, this appointment was confirmed by the Government of India on the understanding that the Bengal Government would permit Cunningham to carry on certain duties attaching to his previous office until the "return of his recent coadjutor Dr. Lewis in December." The professorship of physiology at Calcutta was held by Cunningham from then till the close of his Indian service.

By December 1879 the amalgamation of the Medical and the Sanitary departments had taken effect and Lewis on his return remained the only special assistant in the combined department. In 1883, however, Lewis was recalled to England to fill the post of Assistant Professor of Pathology in the Army Medical School at Netley. From the date on which Lewis departed till 1897 Cunningham occupied the position of secretary to the Surgeon-General in the Sanitary branch of the Medical department, and in this capacity, until he left India, performed the duties that had been entrusted to Lewis, in addition to those connected with the professorship of physiology.

During the few years of intermission in Cunningham's pathological and hygienic investigations the Bengal Government availed themselves of his services in other ways. Thus in 1879, when the late Dr. (afterwards Sir) G. King, F.R.S., was deputed to enquire into the organisation of the Cinchona department in Java, Cunningham was appointed to act as superintendent of cinchona cultivation in Bengal in addition to his college duties. Again in 1880, when the same officer was on leave, Cunningham was once more appointed to take charge of the Cinchona department and to act besides as superintendent of the Calcutta Royal Botanic Garden.

When Cunningham resumed his official pathological work in 1883 a suitable laboratory, built according to his design, was provided for the purpose in the grounds of the Calcutta Presidency General Hospital. Here during the next 14 years, save for a break of several months when he served as naturalist with the Tibet mission of 1886, and during two brief visits to Europe—one of these as a representative of the Indian Government at a convention held in Rome to consider international regulations as to quarantine—Cunningham prosecuted with success these enquiries into tropical diseases which had already proved of such public value.

His duties at the Medical College compelled Cunningham to make Calcutta, with its trying conditions, his research-centre. These conditions were the more severe because his official investigations involved almost unbroken residence and deprived him of the opportunities of change of scene which college vacations are meant to provide. As a consequence his constitution became gradually undermined and in 1897 a severe illness, induced and aggravated by the climate of the Gangetic delta, led to Cunningham being invalided to Europe. His life, which had been in danger, was thereby saved. But his health was permanently impaired, his return to the Tropics was forbidden, and in June 1898 he had to relinquish his Indian career. Later in the same year he settled at Torquay, where, as an invalid, he devoted the rest of his life to his garden and his books.

When the value of Cunningham's official work is considered, account has to be taken of the circumstances which conditioned it and the object which underlay it, as well as the manner in which it was performed. It took, of necessity, the form of studies in applied pathology, unconcerned with clinical needs, but intended to provide a scientific basis for sanitary administration. These studies served their special purpose well and their utility was largely

due to the philosophical spirit which permeated them. They had always a physiological basis, for Cunningham recognised no distinction between physiological and pathological processes, whether in the animal world or in the vegetable. The same catholic outlook characterised his unofficial contributions to natural knowledge—the by-products of his official work and the recreations of his active intellect.

The greater proportion of Cunningham's reports and papers bear directly on the cholera enquiry with which he and Lewis were originally charged, and to which he gave undiminished attention throughout his official career. His pathological contributions, however, include observations on fungus-disease in collaboration with Lewis in 1875, and on Delhi boil and mycetoma published independently in 1885 and 1895. Among questions which attracted his interest as a physiologist or as a consequence of his connection with the Botanic Garden were the action of snake-poison, the subject of papers in 1869, 1895, 1897, 1898; the effects of starvation on vegetable and animal tissues, in 1878; nyctitropism and allied movements in plants, in 1882, 1888, 1895; gaseous evolution from the flowers of *Ottelia* and fertilisation in the genus *Ficus*, in 1887. Among matters where his interest is perhaps traceable to his early intercourse with Berkeley and De Bary were economic studies of diseases in plants, published in 1878, 1896, 1897, and biological studies of parasitic *Algæ* and *Fungi* in 1880, 1888, 1889, 1895.

These papers, however, give a very inadequate idea of Cunningham's work in the field of cryptogamic botany. For about a quarter of a century it was his practice to deal, on behalf of his friend King, with the countless economic references by government officers and private cultivators regarding plant diseases caused by vegetable parasites. So effective was this aid that it was not until Cunningham had retired that the Government of India realised the need for the services of a whole-time plant-pathologist. It is interesting to reflect that, thanks to Cunningham's private courtesy, they were enabled to avoid an outlay of certainly not less than 18,000*l.*

Cunningham's published papers, however, contain but a small proportion of his observations in fields outside that within which his official work lay. As a child, the harvest of the fishing craft of Prestonpans had been to him an unceasing source of interest, and such satisfaction as the fishers themselves could accord to his curiosity only whetted his boyish craving for further information and laid the foundation of a habit of observing natural objects which, during his school and undergraduate days, developed into a keen addiction to the study of natural history. This taste accompanied him to India and from the time of his arrival it was his practice to enter in a carefully kept series of note-books his observations of animate things. The existence of these records was known to, and the notes themselves were always at the service of, his more intimate friends. To these it was, therefore, a source of keen satisfaction when Cunningham's health at length became sufficiently restored to admit of his exercising his literary gift in rendering some part of this accumulated knowledge available in an enduring form to a

wider circle. This was done in two charming volumes, published by Mr. Murray, in 1903 and in 1907.

Throughout his Indian service Cunningham was called upon to take a considerable part in the performance of such public duties as in England are entrusted to men of leisure, but in our great Eastern dependency have to be undertaken, amid their other labours, by hard-worked officials. Early in his career he was appointed by the Governor-General a member of the Senate of the University of Calcutta. During his residence in India he was at frequent intervals a councillor of the Asiatic Society of Bengal. He was for many years one of the trustees of the Indian Museum. More important still were the services he rendered in connection with the Calcutta Zoological Garden.

This institution, whether as regards the extent and variety of its collection or in respect of the management and health of its inmates, holds a deservedly high place among establishments of its kind. Cunningham's interest in natural history had secured him the friendship of the late Dr. J. Anderson, F.R.S., then superintendent of the Indian Museum and long the honorary secretary to the committee of management of the Garden. The keen interest which Cunningham's intercourse with Anderson led him to take in the collection was of a practical kind, and in November, 1878, he was elected by the committee a life-member of the institution "in consideration of his numerous presentations to the garden." In the following year he was nominated a member of the committee of management by the Lieutenant-Governor of Bengal. Five years later he became honorary secretary in succession to Anderson, and this post he only relinquished when, some years afterwards, the Government of Bengal appointed him chairman of the committee. Among the many improvements which marked his connection with the institution none was greater than the provision, on his suggestion and in accordance with his plans, of a research laboratory. The investigations conducted there included, along with many by other workers, his own connected with snake-venom, whose publication in 1895, 1897 and 1898 has already been mentioned.

Cunningham's work as a teacher was characterised by exact knowledge, clear exposition and singular width of outlook. His influence on his pupils was marked and salutary. His constant aim was to train them to use their powers of observation and to overcome the tendency, induced by a prolonged course of literary instruction in a language not their own, to place too much reliance on statements made in books. His influence on those so fortunate as to know him was equally great. A cultivated artistic sense and a fine literary instinct were in him combined with a methodical disposition of his time and great sagacity of judgment. A keen student of philosophy, in which field he was a follower of Kant, and a man of wide reading, especially in the history of art and literature and in that of travel and discovery, intercourse with him was felt to be a privilege. His charm of manner, serenity of disposition and transparent singleness of purpose made him the centre of a circle of devoted friends, who experienced in his company a sense of repose and were able dimly to appreciate his singular ability to gain the confidence of birds and

beasts and his still stranger capacity to overcome without effort the shyness akin to suspicion which the European as a rule inspires in Eastern races whose instinctively higher ideals have not become impaired by contact with the essentially material culture of the West. Probably no contemporary official knew more intimately or was held in higher regard by the Sikh community than Cunningham, who spoke and wrote its language, was learned in its scriptures and beliefs and had won its heart, in his earlier and athletic days, by becoming a redoubtable wrestler in the Sikh style. The devotion to Cunningham of these soldiers of fortune from the Punjab was as sincere as that of the fishermen of the North Sea, who also regarded him as one of themselves and whose affections he had gained when, a lad in the pursuit of natural knowledge, he had shared their vigils and their hardships off the east coast of Scotland. His unsought influence was as manifest among the Lepchas and Bhotias of Sikkim with whom he had come in contact; to be known as his friend was a sure passport to their affection. But no one was less conscious of this magnetic power than Cunningham himself and the simplicity of character to which it was due enabled him to fulfil as few have done the Greek injunction so to order life as "not to be talked about for good or for evil among men."

The value of Cunningham's services to the State was not unrecognised. Lord Dufferin, when Governor-General, of his own motion appointed Cunningham an Honorary Surgeon to the Viceroy, and he remained on the staff of succeeding Governors-General until he retired. In June 1893 he was made a Companion of the Order of the Indian Empire; after his retirement he was appointed an Honorary Physician to the King. In 1876 he was elected a Fellow of the Linnean Society. When he took up the duties of secretary to the committee of the Zoological Garden at Calcutta he was made a Corresponding Member of the Zoological Society; on his retirement from India, he became a Fellow. In 1889 he was elected a Fellow of the Royal Society, and in 1898 his great services to the Calcutta Zoological Garden were commemorated by the presentation to that institution of a bronze medallion portrait provided by his friends.

Cunningham died, after a brief illness, at his residence in Torquay on December 31, 1914, and the friends who, four days later, laid his remains at rest, felt how pleasant a page in the book of their lives had been closed, leaving them a memory which cannot die, to "walk in its whiteness the hall of the heart."

D. P.

HENRY CHARLTON BASTIAN (1837—1915).

Dr. Bastian was one of the oldest living Fellows of the Royal Society, having been elected in 1868. He was born at Truro on April 26, 1837, and died at his residence, Chesham Bois, Bucks, on November 17, 1915. He had a distinguished academic career at the University of London, graduating M.A. 1861 and M.D. in 1866. For a short time he was Lecturer on Pathology and Assistant Physician at St. Mary's Hospital, which post he resigned in 1867 to return to his Alma Mater, University College, as Professor of Pathology and Assistant Physician to University College Hospital. He later became full Physician, and resigned in 1897.

Quite early in his career Dr. Bastian showed the spirit of scientific investigation, and published a monograph on the Anguillulidæ, 1865, of which he discovered 100 new species of one family; also a monograph contributed to the Linnean Society on Nematoids, parasitic and free.

During his appointment as Assistant Physician to University College Hospital his clear analytical mind was directed towards neurological science, and quite early in 1867 he described and pictured in a communication to the Royal Medico-Chirurgical Society a tract in the spinal cord, afterwards known as Gowers' tract. In 1868 he was appointed Assistant Physician to the Hospital for the Paralysed and Epileptic in Queen Square, with which institution he was associated till 1912. He became full Physician in 1887, retired in 1902, and remained a Consulting Physician, with ten beds, till 1912.

During this long period ample opportunities arose for the investigation and study of Nervous Diseases, and his many works are remarkable alike for lucidity of exposition, accurate observation and sound reasoning. Moreover, his works on Neurology reflect his keen critical faculty and knowledge of Psychology. Indeed, in his writings one frequently sees the influence of his friend Herbert Spencer, with whose works he was most familiar. In 1869, Dr. Bastian published in the 'British and Foreign Medico-Chirurgical Review' the first paper of a remarkable series of observations and deductions on the subject of Aphasia, which, if he had done nothing else, would have sufficed to establish for him a great scientific reputation as a pioneer in Neurology. In this monograph, entitled, "On the Various Forms of Speech Defects," he anticipated Wernicke by five years, for he clearly described that affection of speech usually spoken of as "Wernicke's Sensory Aphasia"; but if it is desirable to associate any name with any particular affection or disease it should be "Bastian's Sensory Aphasia." From this time onward he continued to publish many valuable monographs and books relating to Neurological Science. Among the most important are 'The Brain as an Organ of Mind,' International Series, 1882;

'Paralyses: Cerebral, Bulbar, Spinal,' 1886; 'Hysterical and Functional Paralysis,' 1893; 'Aphasia and other Speech Defects,' 1898. These books are perhaps not read as much as they might be, but to neurologists they are a mine of wealth, not only on account of the clinico-anatomical observations made by Dr. Bastian himself, but also on account of the care with which he has selected cases published by others in support of his views, which are set forth with precision of detail, philosophic insight, and literary skill. The author made difficult subjects comprehensible to his readers; and "the forest is never lost in the wood."

The recent war experience of gunshot injuries to the spine causing concussion of the spinal cord proves the truth of the valuable observation of Bastian, who was the first to show that the knee jerks are abolished in total transverse lesions of the cervical spinal cord. No one has set forth more clearly than this author the doctrine of aphasia, and his views on the subject are widely accepted. Although schematic, they seem best to fit in with the facts of cerebral localisation as determined by clinico-anatomical observations. The name of Charlton Bastian will live, with that of two other distinguished Fellows, Hughlings Jackson and Gowers, as founders of Neurological Science in this country.

Dr. Bastian would not himself have accorded to his neurological work the importance that his former pupils and followers have, but would have said that his life work was that upon Abiogenesis.

In 1872 Bastian published 'The Beginnings of Life,' which led to a great and memorable controversy in which Pasteur, Tyndall, and Huxley took part. Bastian's views were not accepted by the scientific world. Still, his experiments showed that some scientific beliefs of his adversaries were not true; it was claimed that boiling would kill all germs, and that if organisms appeared in Bastian's flasks after boiling it was due to faulty methods of technique; but it was subsequently found by Pasteur that desiccated germs in the form of spores could resist boiling. Moreover, Bastian showed that germs can exist in the living body, which we now know to be true; although the inference that they arose by spontaneous generation or by heterogenesis is both unnecessary and unlikely. Dr. Bastian always, however, held an impregnable position when he maintained that living matter must at one time have originated on the earth from non-living matter, and there is no logical reason why this process should not be continuing.

In 1893 Dr. Bastian gave up the Chair of Medicine at University College, London, which he had held for six years, and in 1897 he gave up his post of Physician to University College Hospital, which gave him the leisure he needed to renew his experiments and observations on his life work of abiogenesis and heterogenesis. He learnt the technique of photo-micrography in order that he might represent faithfully his observations and thus convert the biologists to a belief in them, or at any rate to give heed to his experiments and observations, which he published in four volumes of 'Studies in Heterogenesis,' illustrated by 805 photo-micrographs—1905 and onwards.

This work is a monument of patient and honest endeavour to support a lost cause. He felt deeply the rejection of his papers to the Royal Society. One paper he sent January 16, 1902, "Note on the Transformation in the course of three or four days of the Entire Contents of the Egg of *Hydatina Senta* into a Large Ciliated Infusorium belonging to the Genus Otostoma," having been rejected, his comment was: "Were the announcements in this paper new or were they true? That they were new, there could be no doubt—their truth could only be gainsaid by investigation of the specimens."

In November, 1912, Bastian read a paper before the Pathological Section of the Royal Society of Medicine, and claimed that elementary organisms will appear in sterilised fluids; these elementary organisms are found to be associated with well-developed Torulæ or with bacteria, which can be shown in the course of a few days to be living by the growth and multiplication which they undergo under the conditions indicated on p. 59 of the 'Origin of Life.' Precisely similar associations with growing Torulæ were seen and described in 'The Beginnings of Life,' pp. 281–283. He claims that the crucial point of discussion, namely, the introduction of germs by experimental error, or of desiccated germs not having been sterilised by heating, has been put out of court. This is the essential crux of the whole question. Is there a fallacy in the technique? The possible explanation is that there was a partial vacuum in his tubes, and that when he opened them air containing such germs as Torulæ and other moulds (which are very common) entered and vitiated the experiment. So far no scientist of repute has confirmed his experiments, but he would have asked, Have they been tested? It might happen that, without confirming Bastian's doctrine of heterogenesis, new facts might be discovered, as was the case when it was found that desiccated germs resisted boiling.

Notwithstanding the fact that 30 years ago the question of "spontaneous generation" was dead to the scientific world, one cannot but admire the courage and honesty of conviction with which Dr. Bastian with such tenacity of purpose and long patient research endeavoured to re-establish his position. But the technique of bacteriologists had made enormous advances in those 30 years and, however careful a worker is, without laboratory experience and training, unforeseen errors are likely to occur.

As to the origin of living matter most scientists will agree that there is a difficulty in refuting the following statements by Bastian:—

"When it is said, therefore, that a belief in spontaneous generation would tend to contradict the experience of all mankind, my reply is: That archibiosis *may* be occurring all around us, and that from its very nature it must be a process lying altogether outside human experience and never likely to come within the actual ken of men."

"To ask a person to believe that all forms of life on this planet have during countless ages evolved from the primordial living matter which first appeared thereon, and at the same time to ask him to believe that the causes of this evolution through all these ages have been inoperative upon myriads

of the most primitive and modifiable forms of life, has always seemed to me, in spite of the attempts at explanation that have been made, most strange and unreasonable."

It is remarkable how sometimes men of great intellectual capacity and scientific attainments pursue an idea, regarded by others as an *ignis fatuus*, ever leading on yet never reaching the goal.

Whatever view may be taken of Dr. Bastian's position of eminence as a biologist, neurologists will know him as one of a group of pioneers and founders of their branch of the science of medicine. That he was so honoured is shown by the honorary degrees bestowed upon him. He was Fellow of the Royal College of Physicians of London, and was a Censor in 1897–8. He was Honorary Fellow of the Royal College of Physicians of Ireland and was granted an Honorary M.D. by the Royal University. He was a corresponding member of the Royal Academy of Turin, and of the Medico-Chirurgical Society of Bologna and an Associate Member of the American Neurological Association. For many years (1884–1898) he was Crown Referee in cases of supposed insanity. In June last he was awarded a Civil List Pension in consideration of his services to science.

He married Julia, third daughter of the late Charles Orme, and had three sons and a daughter. His widow survives him. One of his sons is Staff Surgeon in the Royal Navy.

F. W. M.

ARTHUR SHERIDAN LEA (1853–1915).

ARTHUR SHERIDAN LEA was born in New York on December 1, 1853. He was the son of Mr. J. Lea by his wife, Susan Sylvia Hobbs. His father, who lived for some years in America, returned to England and settled at Liverpool. He sent his son in due course to the Royal Institution School of that city. In 1872 Lea entered at Trinity College, Cambridge, coming up to the University with a school scholarship in Classics and Mathematics. He became a Scholar of the College in 1875, took a First Class in the Natural Sciences Tripos in the same year, proceeded to the M.A. degree in 1879, and took the Sc.D. degree in 1886.

A short time after taking his Bachelor's degree, whilst he was still undecided what career to follow, two suggestions were made to him: one that he should become a Master at a Public School, with a probability of a House later on, the other—by Dr. Foster—that he should devote himself to higher teaching and research in Physiology. Lea chose the latter, in spite of its meagre pecuniary prospect. In 1881 he was appointed Lecturer in Physiology and Assistant Tutor of Gonville and Caius College, in 1885 he was elected Fellow of the College, and in 1886 Bursar; the latter post he only held for two years. In 1884 he became Demonstrator of Physiology and University Lecturer.

Lea's special interest was in Physiological Chemistry. This study, as it was carried on in England in the '70's and '80's, did not involve any profound chemical knowledge; it was mainly concerned with the experimental determination of simple but fundamental reactions of the fluids and tissues of the body. Lea's knowledge of chemical theory and methods was more than adequate for the conditions of the time, and to him was due the establishment of an advanced course of Physiological Chemistry at Cambridge which enabled students to keep abreast of the growth of the subject.

In the early editions of Foster's 'Text-book of Physiology' the chemical aspects of the subject were given in an Appendix, which was mainly written by Lea. In the 5th Edition the Appendix was much enlarged by Lea and issued as a separate volume of the Text-book, under the title, 'The Chemical Basis of the Animal Body' (1892).

Lea's earliest investigation was carried on with Kühne in Heidelberg. One may conjecture that Kühne suggested the work and that Lea overcame its practical difficulties. Like many other scientific workers, Lea liked the methods of research, and was never more contented than when busy in the workshop. Kühne and Lea examined the living pancreas under the microscope and described the changes in appearance which occurred in it during secretion. Little if anything has been added to their descriptions in the

37 years since it was published. They described also the peculiar arrangement of the blood-vessels in the islets of Langerhans.

In Physiological Chemistry Lea's research was of a somewhat fragmentary nature. This, no doubt, was partly due to illness, which broke the train of his thought.

In 1883 he, in conjunction with Green (then a beginner), published some observations on fibrin-ferment. Their chief conclusion was that fibrin-ferment was not a globulin. But they pointed out that certain facts were not easily explained on this view, and they proposed to investigate them later. Lea gave up the investigation. Green carried it on and it led to the first big step towards our knowledge of the part played by calcium salts in coagulation.

Most of Lea's other papers were also concerned with the action of ferments. In conjunction with Dickinson he showed the untenability of Fick's improbable theory that the mode of action of clotting ferments was fundamentally different from that of ordinary digestive enzymes.

In 1885 he extended Musculus' observations on the isolation of urea-ferment from the organism of decomposing urine. This work was an early recognition of the importance of intra-cellular enzymes.

In 1900 he introduced the method of observing the effect of digestive ferments in dialysing tubes kept in constant movement in running water, so that the products of digestion were removed almost as soon as they were formed. Whilst later discoveries as to the fate of the bodies removed by dialysis showed that Lea's method was a very partial imitation of the natural digestive processes, he brought out clearly the influence of the removal of digestive products on the rapidity of ferment action, and his general views, though not in accord with what is now known, served at the time as a stimulus to a broad consideration of the relation of digestive products to metabolism. He published also a paper on spectrophotometry.

Lea suffered from a progressive spinal disease which early interrupted his work and eventually compelled him to abandon it. For some years he led an active outdoor life. He was captain in the Volunteer Force, went to Aldershot for special courses on Army Supply, and regularly took part in the competitions of the National Rifle Association at Wimbledon. He had marked musical taste, inherited from his father, and was one of the founders and the first Treasurer of the Cambridge Musical Club. More or less of the Long Vacation he spent in cruising round the coast. These and other activities were gradually curtailed, and in 1899 he left Cambridge and retired to Sidcup in Kent. There he still followed, to a certain extent, the development of Physiology, and corrected the proofs of the later editions of Huxley's 'Lessons in Physiology' and Smith's 'Veterinary Physiology.' He died quietly on March 23, 1915. He left a wife and one son. It was with Lea's ardent approval that his son, at the outbreak of war, at once applied for and obtained a commission in the army.

Lea had to submit to seeing his early promise of high scientific activity

dwindle away. He had to submit to the gradual loss of capacity to carry on the occupations of his leisure. He was one of the most companionable of people, and he had to submit to be deprived of much of the companionship of his friends. He bore all this uncomplainingly and indeed with almost constant cheerfulness. His character had a fine touch of fortitude that commanded the admiration of all.

J. N. L.

AUGUST FRIEDRICH LEOPOLD WEISMANN, 1834–1914.

AUGUST FRIEDRICH LEOPOLD WEISMANN was born January 17, 1834, at Frankfort-on-the-Main. His father was Professor of Classical Philology at a "Gymnasium," his mother a refined woman with considerable talent in music and painting. Weismann, the eldest of four children, looked back upon a simple happy home life and always spoke with great admiration of his parents, holding them up as examples to his own children.

Weismann's supreme interest in natural history was evident in early boyhood. He tells us that when he was about 14 his mother asked him what he intended to be, and, when he was unable to give an answer, she went on: "I know what you will be, but I will not tell you lest it should never come to pass." Nor could he persuade her to say any more. Much later, long after her death, Weismann understood and knew that she had recognised the naturalist in him. She had observed the eagerness with which, as a child, he had collected butterflies and bred caterpillars, and how he was inspired and charmed by all living things.

Weismann's father did not altogether approve of absorbing interests which naturally tended to draw a schoolboy away from his work. But he did well as a student, and his father, content with a warning, allowed the collecting to go on. After butterflies and moths Weismann took to beetles, and then to plants. Toward the end of his school days his herbarium contained nearly all the higher plants, including the grasses, which grew wild within a 10 hours journey of Frankfort.

When the time came to decide upon a career many of Weismann's friends thought that he was intended by nature for a botanist, but his father considered that he ought to choose some subject with better prospects of making a living. About this time Weismann attended the lectures given by Prof. Böttcher to the Senckenberg Society in Frankfort and was inspired to become a chemist. But Wöhler, who in the holidays often returned to his

native town, advised Weismann to follow the course which he had himself pursued, first studying Medicine and leaving Chemistry as a later possibility. So Weismann began in 1852 the four-years medical course at Göttingen, and he always afterwards recognised that the Anatomy and Physiology there studied formed an essential foundation for the Zoology to which his life was devoted.

Weismann next became for a year (1856–7) an assistant in the clinical hospital at Rostock and then for another year assistant to the Rostock chemist Franz Schulze. Here he came to realise that Chemistry, in spite of his strong inclination towards it, was not the science for which he was born. He felt not only that he lacked many of the qualities essential for the successful chemist, but that he possessed others, such as the sense of form and order, which were unessential. In 1858, after visiting Vienna, he returned to Frankfort and began to practise as a physician, occupying his abundant leisure in histological research. In 1859 he entered the southern German army as a surgeon and travelled in Italy, tending the wounded in the war which had just been brought to an end by the battle of Solferino. His travels in this year took him to Genoa, where he first met his future wife. Returning to Frankfort towards the end of 1859 he soon tired of practice and accepted the post of private physician to the Archduke Stephan of Austria, but before taking up his residence at Schaumburg Castle on the Lahn, he studied (1860) Zoology in Paris at the Jardin des Plantes, and above all spent two months under Rudolph Leuckart at Giessen. Weismann always felt that these two months were among the most inspiring of his life. During his two years (1861–2)* with the Archduke he had plenty of leisure and was able to begin and in all essentials to complete his first great work—on the development and metamorphosis of insects.

Weismann now considered that the time had come when he might offer himself to a German University. On his 70th birthday he gave the reason for his choice:—“When I first visited Freiburg, in the year 1860, the quiet town, nestling among green vines, with its lime-planted rampart, the clear streamlets in the streets and the splendid Minster, made such a charming impression upon me that I thought, ‘If only one could live here!’ And when, three years later, the question arose of attaching myself to some German University, my thoughts naturally turned to Freiburg.” He joined the University of Freiburg-im-Breisgau in 1863 as Privat-Docent in the Medical Faculty, teaching Zoology and Comparative Anatomy, which had been previously undertaken as secondary subjects by Prof. Fischer, the Mineralogist. There was no proper Department of Zoology—only a single room containing the scanty collections. But Weismann's energy and success soon led to improvements, and in 1886 a Zoological Institute and Museum were built. In 1866 he was elected Professor Extraordinarius, and in 1874† Professor Ordinarius, being the first occupant of the Chair of Zoology in the

* The years 1861–1863 are also given.

† 1871 and 1873 are also given; 1874 appears to be the most probable.

University. He retired April 1, 1912, but still continued to reside in Freiburg.

The happiness of freedom to work at the science he loved was soon clouded by a terrible menace. In the summer of 1864 Weismann's eyes failed him. He suffered as the result of overstrain from hyperæmia and great sensitiveness of the retina, which continued for a long time to increase and seemed about to end his whole career. The loving care and continual help of his wife, whom he married in 1867, helped him to bear the burden and prevented him from giving way to despair. He finally sought and obtained leave of absence from duty for two years (1869–71), determined to avoid all reading and straining of the eyes. He spent the winter of 1869–70 with his family in Italy, and there an improvement began and continued on his return, so that he was able to resume his lectures after the interval, and, in 1874, to return to his researches. He speaks with enthusiasm of the joy of renewed work. "I cannot tell you what happiness these labours brought me. It was the greatest pleasure imaginable to sit again at the microscope and study the minute inhabitants of the fresh waters or of the sea in all their wonderful artistic beauty." Weismann also knew well that it was of the utmost importance—and especially for himself with his keen interest in general questions—to provide by a wealth of precise and detailed knowledge the only secure foundation for philosophic thought and speculation.

After ten years of microscopic research his left eye broke down, becoming not blind but unfit for further work. But by this time he had laid a firm foundation of knowledge and had attracted a band of able pupils through and with whom he could continue to work without endangering the power of his right eye. Chief among those who helped him with the microscope was one of his most gifted pupils, Ischikawa, the eminent Professor of Zoology at Tokio.

Weismann felt that his life had been controlled and directed by the power of ideals, that power which, in spite of the interference of chance, makes a man strive to use his strength where it can achieve most—in fact to strike and keep the right path in life. He felt that it was certainly not by chance that he gave up Medicine and forsook Chemistry and Botany after having studied them—it was the power of ideals leading him to make the best use of the capacities with which he came into the world.

Weismann's friends will recall his fine intellectual head, with the tired strained look brought on by the trouble with his eyes. He was tall and strongly built, very fond of walking, and devoted to the country. The love of living things, noticed by his mother in his boyhood, was strong in him throughout life. Weismann was not much of a traveller; once only he left Europe, and then but for a few hours, when he crossed the Bosphorus to Scutari to enter the celebrated cypress forest, and, from the heights behind the town, to look down on the marvellous pageant of Constantinople.

As a companion he was charming and sympathetic, possessed of a noble simplicity. To younger men he was ever generous and kindly, and many in this country will remember the encouragement received from his kindly appreciation.

Weismann found intense delight in the world of art. On his visits to Italy he studied with the keenest pleasure the works of the great masters. He was probably even more devoted to music. "His memory was wonderful, and he was able to keep up his playing of Bach's works until his eightieth year, although his fingers almost refused to do their work, and his sight nearly failed him." With Huxley he might have quoted the beautiful lines of Landor:—

> "Nature I loved, and, after Nature, Art.
> I warmed both hands before the fire of life;
> It sinks and I am ready to depart."

The words are singularly appropriate to the great naturalist, who "passed away peacefully . . . while his son was playing some of his favourite music."

In 1867 Weismann married Mary Dorothea, the eldest daughter of Adolf Gruber, of Genoa. The second daughter afterwards married another Professor at Freiburg, Robert Wiedersheim, while the eldest living son, August, studied Zoology, and also became a professor at the same University. In summer vacations the large family used to meet at Adolf Gruber's beautiful estate on the Lake of Constance, and here Weismann pursued his researches on the fresh-water fauna.

Weismann's family consists of four daughters and one son, the youngest of the family, who was only four years old when his mother died (1886). His second daughter is married to Professor W. N. Parker, of Cardiff. Both Weismann and his wife were very musical, and the gift is inherited in a higher degree by his son, Julius Weismann, who takes a prominent place among the younger composers. His playing was a constant source of joy to his father.

Weismann once asked the present writer what he thought of Bismarck and his policy. This was before the day when a wretched and bitter old man strove to magnify his own importance by boasting of forgery, and the world was startled to learn that, while condemning folly, it had extended its sympathy to crime. Would that it had been otherwise; for it is of the highest interest to know what such a German as Weismann would have said of war deliberately provoked, and provoked by the methods of Bismarck. At the time he considered the policy good, but talked as if he was quite aware that there was much to be said on the other side. On his seventieth birthday he spoke of the great national elevation of the Franco-German War, and of how the "unexpected new birth of our fatherland, filling us all with joy," perhaps contributed towards the cure of the essentially nervous trouble with his eyes. Weismann and his family had never liked the Prussians or

Prussian militarism. It was the German victory he rejoiced in, and above all the united Germany which sprang from it.

Weismann was among those who publicly renounced the marks of distinction which had been conferred on them in this country. A few weeks before the war broke out, his daughter, Mrs. W. N. Parker, her husband, and little girl, went to stay with Weismann, near Lindau, on the Lake of Constance. They found him much aged, and were rather anxious about his heart, though he was able to take short walks and to enjoy his daughter's playing, and even, in spite of his increasing blindness, to do some work on the colours of butterflies, the subject of his last letters to the present writer. The war news excited and upset him very much, and they decided to remain with him until they could be replaced. Julius appeared on Sunday, August 2, and the next day they thought it advisable to cross to Switzerland, and were only just in time. Professor and Mrs. Parker felt that, if they had been able to stay with him, Weismann would not have signed the memorial. It is known that for a long time he refused to sign it, and only gave way to persistent persuasion. But, excited and unwell, and with all the sudden anxiety about Julius and so many other members of the family, it is hardly to be wondered at that he was not in a state to judge calmly and fairly; and we may be sure that he did not know the truth about England's participation. We can imagine, too, how terribly grieved a man of his affectionate disposition must have been at the knowledge that members of his family were ranged on opposite sides. Weismann's many friends and admirers in this country will feel the force of these considerations, and will realise the effect of such a sudden and awful shock upon a man of over eighty and in failing health.

The outbreak of war brought on an attack of heart trouble, but he recovered sufficiently to return to his Freiburg home, where he was tended by his eldest daughter. He began to lose strength about the middle of October, and his son, who was engaged at the base hospital at Lindau, obtained leave to go to his father. Weismann sank gradually, and died without pain on the evening of November 5, 1914. His age was eighty years and nine months.

It would be inappropriate to describe or discuss at any length the splendid series of memoirs by which Zoology was enriched by this great man, and I therefore reproduce the brief account of his scientific labours, drawn up for 'Nature' (vol. 94, 1914, p. 342).

"Weismann's earliest researches were physiological and histological, the first publication, on hippuric acid (1858), being followed by a series of six papers on the nervous and contractile tissues (1859–1862). Abandoning this subject, except for a single paper on muscle, published in 1865, he threw himself with the utmost energy into his classical work upon the embryonic and post-embryonic development and metamorphosis of insects, producing five memoirs between 1862 and 1864 and a sixth in 1866. In the great monograph on the post-embryonic development of Muscidæ (1864), the

building up of the perfect form in the pupa is studied in detail, and it is shown that, in insects with a complete metamorphosis, the tissues undergo a breaking down or histolysis into an apparently simple and primitive mass, from which the imago is built up afresh by, as it were, a second embryonic development. Thus the long series of slightly modified progressive steps by which, in the more ancestral groups, the earliest stage is transformed into the latest has been shortened in the more recent forms into a single intermediate stage, in which everything is broken down and built up again from the beginning, establishing the truth of Aristotle's statement that 'the chrysalis has the potentiality of the egg.'

"Insect development was followed by a great series of memoirs (1874–1880) on the minute Crustacea—Daphnids and Ostracods—and these again by the epoch-making researches into the sexual cells of the Hydrozoa, published in four papers between 1880 and 1882, and, in 1883, in the great quarto monograph, 'Die Entstehung der Sexualzellen bei den Hydromedusen.' With the appearance of this work Weismann's eyesight became too weak for prolonged microscopic research, and he turned to other and more general problems of thought and inquiry.

"Weismann was attracted early in his career towards the problems of the history and causes of evolution. 'The Origin of Species' appeared in the year following the publication of his first paper, and in 1868 he brought out 'Ueber die Berechtigung der Darwin'schen Theorie,' followed in 1873 by his paper on the influence of isolation, written in answer to Wagner. The 'Studien zur Descendenz-Theorie' (1875) included a variety of subjects treated from the evolutionary point of view—the seasonal dimorphism of butterflies, the markings of caterpillars, phyletic parallelism, the transformation of the Mexican axolotl, and the mechanical conception of nature. This important and stimulating work, translated into English, with many additional notes by Raphael Meldola, and with a preface by Charles Darwin, was published in 1882. The present writer well remembers the interest with which he looked forward to the parts as they successively appeared and the instant resolution to continue some of the lines of work.

"The central thought which branched forth so luxuriantly during the last 30 years of Weismann's life sprang from his researches on the sexual cells of the Hydrozoa. By these he was led to conclude that, however ordinary their appearance, the germ-cells contain something essential for the species, something which must be carefully preserved and passed on from one generation to another. It was this conclusion, so Weismann told the present writer in 1887, which led directly to the hypothesis of 'The Continuity of the Germ-plasm,' with all its far-reaching consequences. In Darwin's pangenesis the germ-cells are derived from the body-cells, whereas in Weismann's contrasted hypothesis the body is an outgrowth from the germ. From this conception Weismann was led to contrast the mortal soma with the potentially immortal germ and to question the hereditary transmission of acquired characters. Excluded from the Darwinian interpretation of germinal variation as a

consequence of gemmules dispatched to the germ by environmentally modified body-cells, Weismann looked for the origin of variation in the kaleidoscopic combination of innumerable ancestral factors brought about by sexual reproduction. He thus sought to explain the meaning of sexual reproduction itself as well as the events which lead up to the fusion of the male and female germ-cells.

"The subjects thus briefly enumerated, treated in eight memoirs published between 1881 and 1888, were translated, and appeared in a collected form in this country as 'Essays upon Heredity and Kindred Biological Problems' (1889). The translation of four additional memoirs (1886–1891) was published as a second volume in 1892, the year in which he produced The Germplasm,' translated by Prof. W. Newton Parker and published in this country in 1893. An elaborate and remarkable hypothesis, 'Germinal Selection' (1896), was followed by the comprehensive treatise on the evolution theory, which brought his long and fruitful life-work to a close. The two volumes passed through three editions between 1902 and 1913, the English translation by Prof. and Mrs. J. Arthur Thomson appearing in 1904, the year of the Festschrift which celebrated Weismann's seventieth birthday."

It is truly said in the Academical Vade-mecum (1904) of his University that Weismann considered his chief duty as a teacher was to spread the doctrines of Darwin, and that the aim of all his investigations was to confirm and further develop them.

In these years of revelation it is well to be reminded of the words of warning and advice to younger men uttered by Weismann on his seventieth birthday:—

"You will not be among those thoughtless people who believe that the main object of science is to make useful discoveries . . . . steam engines, telegraphs, telephones, antiseptic treatment of wounds, knowledge of fever parasites in the blood, and the like. However important these and many other advances are for humanity, they would never have taken place at all if research had looked to them as its sole object. They are but by-products of the investigator's workshop, where the real aim must always be pure knowledge for its own sake, unbiassed by secondary efforts. Antiseptic treatment could scarcely have been discovered without a previous knowledge of the lowest and smallest of known organisms, the bacteria, and the life-histories of many harmless forms. Or how could we ever have come to know of the parasites of malaria living in the blood if we had not previously studied similar lowly forms of life inhabiting water and other media? And these studies were not pursued because it was foreseen that they would one day become splendidly important for us, any more than Weber and Gauss studied electromagnetism in order to invent the telegraph . . . ."

It is fitting to conclude this brief story of Weismann's life with the true and noble thought contained in his address at the Darwin Centenary

celebration in Freiburg. "It is a perversion of the theory of evolution to maintain, as many have done, that what is merely animal and brutal must gain the victory. The contrary seems to me to be true, for in man it is the spirit that rules and not the body."

Note.—The material from which this brief account of Weismann's life has been written has been almost entirely drawn from the four publications quoted below. I have tried to express in my own words, and only rarely by a translation—and then with extreme freedom—what I believe were Weismann's thoughts about himself and his career. I could not have attempted the work but for the kind help of my friends Prof. and Mrs. W. N. Parker and Mr. E. A. Elliott.

The Obituary Notice in the 'Proceedings of the Linnean Society,' Session 127, 1914–15, pp. 33–37, by Professor and Mrs. W. N. Parker.

The Obituary Notice in 'Nature,' vol. 94, pp. 342–343, November 26, 1914, by E. B. Poulton.

'Akademisches Vademecum für die Albert-Ludwigs-Universität, Freiburg i. Br.,' Herausgegeben von Hans Speyer. Freiburg i. Br., 1904.

'Bericht über die Feier des 70 Geburtstages von August Weismann, am 17 Januar 1904, in Freiburg i. Breisgau.' Jena, 1904.

E. B. P.

Sir WILLIAM TURNER, K.C.B. (1832–1916).

The death of Principal Turner has removed from the roll of the Society the name of one who occupied for more than half-a-century a very prominent place among anatomical teachers and medical legislators.

Although so long associated with Edinburgh University, he was English by birth, having been born in Lancaster in 1832. His father was in business in that town, and died when his son was five years old. His mother (who was a Scotswoman, maiden name Aldren) sent the boy to a private school in Lancaster kept by Mr. Howard, and, when he was 16, apprenticed him to a local surgeon, Christopher Johnson. From him he learned a little chemistry, a subject in which he retained his interest all through his life.

Two years later he began his more serious medical training at St. Bartholomew's, where he obtained a scholarship. He attracted the notice of Sir James Paget, then assistant surgeon, by his intelligence and his interest in the study of pathology. Through him Turner made his first public appearance, as in 1853 Paget communicated for him to the Royal Society a paper on "The Examination of Cerebrospinal Fluid," which showed a very considerable knowledge of chemistry. This was published in 'Proceedings,' vol. 7, p. 89. In the same year he took honours

in chemistry at the London University, and obtained his M.R.C.S. qualification.

While working at St. Bartholomew's, the place and nature of his future life-work was determined by an unexpected occurrence. In 1854 Prof. John Goodsir, of Edinburgh, then in infirm health, when passing through London, returning from a sojourn at Wildbad, called on Paget and consulted him as to the choice of a demonstrator to assist him in the anatomical classes in his University. Paget at once suggested Turner, and, after a brief characteristic interview, Goodsir appointed him as his assistant on the spot.

Turner entered upon his new duties in the winter session of 1854. At first he found his surroundings somewhat uncongenial, but his good temper, sagacity, and straightforward earnestness soon disarmed all strained relationships with those who had resented the intrusion of a stranger, and in a very short time the young teacher earned the good will of all with whom he was associated. He thoroughly adapted himself to his environment, and before long was strong enough to have his own way in his work. It was his duty to give systematic anatomical demonstrations, to supervise the practical work in the dissecting room, and to continue the demonstrative teaching of histology, which Goodsir had started some years before, with the aid of Turner's predecessor, Drummond.

For some years the routine teaching occupied his whole attention, but, by the time that he was established in his work, he began to publish the records of his anatomical researches. Some of the earliest are descriptions of anomalous conditions found in the dissecting room, but some were records of work of more practical value. Two of these earlier papers were especially important: "On Anastomotic Vessels Connecting the Parietal and Visceral Branches of the Abdominal Aorta," and "On a Supplementary Vascular Supply to the Nutrient Arteries of the Lungs." He also began his studies on cranio-cerebral topography and on the cerebral convolutions, which he expanded later into the larger papers on the subject, which are known to all anatomists. During his service as demonstrator he had as his students, and afterwards as junior colleagues, Cleland, Traquair, Chiene, Morrison Watson, and others, who afterwards filled important teaching posts in Edinburgh and elsewhere.

Goodsir died in 1867, and by that time Turner had been recognised as the most distinguished anatomical teacher in Scotland, so his election to the Chair was a foregone conclusion. Those were the bad old days, when candidates had to furnish the electors with volumes of testimonials. Few such lists ever equalled in weight and warmth those that Turner sent in. The list included letters from all the leading authorities whose testimony was of moment. Two rival candidates also sent in applications, but Turner was unanimously elected. He was Professor of Anatomy for 36 years. In this capacity he took the elementary courses for those beginning the subject, and in this work he was *facile princeps.* His slow, deliberate utterance, well chosen, clear and graphic mode of description, together with the living

interest which he threw into his work, all conspired to make him a popular and successful teacher.

In his first year as Professor, the 'Journal of Anatomy' was started by him in collaboration with Sir G. M. Humphry, of Cambridge. In the first volume the names of Newton, Perceval Wright, and J. W. Clark appear as co-editors, but these merely ornamental names were dropped in the second volume. The two editors at first divided the work between them, with the aid of Prof. McKendrick on the physiological side, but gradually Humphry relinquished his share of the editorial work, and by 1875 the whole of the responsibility was thrown on Turner, although Humphry's name appeared on the title page until his death in 1896. In 1897, Turner associated with himself three new colleagues, to whom he committed the active editorial duties, but to the end he retained his post as senior editor, and every sheet of the Journal passed through his hand until the end of 1915.

During his tenure of the professorship his activity in anatomical research was extraordinary. He produced over 200 memoirs on anatomical subjects, all of which were records of strenuous original work in very diverse branches of the subject. He had been led early in his studies to observe the varieties of placentation in different groups of mammals and, between 1871 and 1889, published a large number of valuable papers embodying his observations. On this subject he gave lectures at the Royal College of Surgeons in 1875–6, which were published as a volume.

In 1874 he began his studies on the anatomy of Cetacea and produced a number of interesting and important memoirs which are painstaking and valuable additions to the literature of a difficult subject. As, through the agency of his pupils, scattered throughout the world, he was able to procure crania of many races of men, he published on these a number of monographs, the earliest of which appeared in 1866, and the last, on Scottish crania, as late as 1915. One of the most interesting of his anthropological memoirs is one on the classification of the races of mankind by the varying forms of the pelvic brim. The observations on which this was based are published in his account of the human remains obtained during the "Challenger" Expedition, upon which he wrote a monograph (Vol. XVI, Part 47). In the same series he also wrote the account of the Cetacean bones (Vol. I, Part 4).

Turner early became known among his colleagues as a cautious, firm, and capable man of business, and in 1873 he was elected by the Universities of Edinburgh and Aberdeen as their representative on the General Medical Council. In this position he gained the well-merited reputation of being a wise and prudent councillor who took a statesman-like view of the subjects with which the Council was concerned. When the Presidency of the Council became vacant by the death of Sir Richard Quain, he was elected to fill the office, and as Chairman, he introduced many measures which increased the usefulness and efficiency of the Council. During the years 1878–80, he was Dean of Faculty in his University, and in 1881 he served on the Medical Acts Commission.

In 1903 he was elected Principal and Vice-Chancellor of the University in which he had worked so faithfully and well for nearly half a century, and in his new position he continued to promote, not only the success of the Medical School, but also the wider interests of the University. The Anatomical Museum and the practical classes in Anatomy and Physiology had greatly outgrown the buildings in the Old University, and it is to his exertions and influence that the University owes the large new buildings which they now occupy, as well as the spacious "McEwan Hall." These buildings cost £228,000. Turner also took an active part in framing the regulations under which Mr. Carnegie's generous gift to the Scottish Universities is at present administered.

In his earlier years he was an enthusiastic Volunteer and retired from the service in 1890 with the rank of Lieutenant-Colonel and the V.D. On the outbreak of the present war he gave all the aid in his power to the promotion of recruiting among the University men in Edinburgh.

He was recipient of many honours. Knighted in 1886 and created K.C.B. in 1901, he also received the Prussian Order *Pour le Mérite*. Elected Fellow of the Royal Society in 1877, he served on the Council in 1890–1, was President of the Royal Society of Edinburgh, and of the British Association (at the Bradford Meeting, 1900); Honorary Member of the Royal Irish Academy and of the Anthropological Societies in Paris, Petrograd, Rome, and Berlin. He took much interest in the founding of the Anatomical Society in 1887, and was its President in 1891–2. He had also received a large number of Honorary degrees, LL.D. of Aberdeen, St. Andrews, Glasgow, Montreal, and Pennsylvania; D.C.L. of Oxford, Durham, and Toronto; and D.Sc. of Dublin and Cambridge.

In 1863 he married Agnes, eldest daughter of Abraham Logan, of Burnhouse, Berwickshire, who pre-deceased him, and he leaves three sons and two daughters. His eldest son is Dr. William Aldren Turner, Physician and Lecturer on Neurology at King's College Hospital; his second son is Dr. Arthur Logan Turner, Consulting Surgeon and Lecturer on Diseases of the Ear and Throat at the Royal Infirmary of Edinburgh and in the University.

He had enjoyed good health throughout his long life, and in spite of his venerable appearance, he was active and able to attend to his duties almost to the last. For some years he found it advisable to winter abroad, but since the outbreak of the war this was impossible. On February 3 he attended a meeting of the Senatus, but feeling unwell he had to leave before the meeting was concluded. The weakness gradually increased and he died quietly in his sleep on February 15.

He has left behind him a threefold record of success: as a teacher, as an investigator, and as an organiser and man of affairs. As a teacher he held a unique record as an educator of anatomical teachers. Nineteen of his students became teachers of anatomy or of kindred subjects in different parts of the Empire. Their great success was due to the encouragement

which he gave to those of his students in whom he perceived the aptitude for research. He knew all the good men in his class and stimulated them to work. He also took an active interest in securing teaching posts for them.

As an investigator he was painstaking and germinal; in most of the work done by his pupils there is discernible the development of ideas which had their roots in his teaching. As a medical statesman his record is honourable, and as a man his death will be felt as a real personal loss by the thousands of his pupils scattered in all parts of the world.

A. M.

EDWARD ALFRED MINCHIN, 1866–1915.

By the death of E. A. Minchin at the age of 49 years on September 30, we have lost one of the most brilliant and most industrious of the zoological investigators of our generation. Though he seemed to choose for his special researches some of the most difficult and intricate problems that his science afforded, he took a delight in training his hand and eye to overcome the greatest of technical difficulties and in opening up new lines of research for himself and his successors. His patience and care in minute details, his firm grasp of the general principles of the subject he had in hand, and his determination to be satisfied only with the very best work he could produce, are evident in all his writings. His devotion to science and untiring energy are shown in the number of books and scientific papers that constitute his life's work.

He was born at Weston-super-Mare in 1866. In his early years he suffered from a constitutional weakness and a physical disability which prevented him from following the ordinary pursuits of a boy's life. At 14 years of age he joined his parents in India and was sent to the Bishop Cotton School at Bangalore, and it was during the years that he spent in India that he was able to indulge freely in his favourite pursuits in Natural History and to develop his powers of observation.

On returning to this country he gained an Exhibition at Keble College, Oxford, and began to study for Honours in Natural Science. During his undergraduate career the Zoological Department of the University underwent several rapid and unsettling changes as regards its teaching staff, owing to the serious illness of Prof. Moseley, but notwithstanding the disadvantages which he suffered in this respect he took his degree in 1890 with first-class honours in Zoology.

As a student he had already distinguished himself by his skill in technical work, by his dogged perseverance and by his marked capacity for original observation. When working over the types given to the senior students for examination he was not contented simply with the confirmation of the facts of anatomy as stated in the text-books, but was willing to devote his spare time to inquiries on points of structure about which he could get no information either from the literature or from his teachers. In this way he discovered the skin pouches—regarded as stink glands—under the fifth tergum of the cockroach, and the brood pouches in the manubrium of Aurelia. He published two short scientific papers on these subjects before he took his degree.

Oxford soon recognised his merit and he was awarded the University Scholarship at Naples, the Radcliffe Travelling Fellowship and finally a fellowship at Merton College. In order to pursue his original investigations he visited the marine biological stations at Naples, Banyul, and Roscoff, and also spent some time at work in the laboratories of Prof. Bütschli at Heidelberg and of Prof. R. Hertwig at Munich. But in 1899 his wanderings abroad were, for a time, brought to an end by his appointment to the Jodrell Chair of Zoology in University College, London.

During this period of his career the principal subject of Minchin's study was the structure of the calcareous sponges and, in particular, the histology and development of the spicules. The memoir entitled "Materials for a Monograph of the Asconidæ," published in the 'Quarterly Journal of Microscopical Science' in January, 1898, was a model of a carefully-written treatise, a clear exposition of new facts, and a philosophical discussion of conflicting views. The important results of Minchin's work on the formation of the triradiate and quadriradiate spicules of the Clathrinidæ led to further researches on spicule formation in Sponges and other groups of animals, both by himself and by his pupil, Dr. Woodland. But Minchin's interest in the Porifera extended far beyond the question of the structure and development of the spicules. He was anxious, if possible, by the study of their general morphology, to solve the difficult problem of the relation of Sponges to other groups of animals. In 1896 he communicated to the Royal Society an important little paper on the larva and post-larval development of *Leucosolenia variabilis*, in which he described the significant fact of the differentiation of the dermal layer of the larva into the outer epithelium and the inner connective tissue of the adult. The result of these investigations led him to two very definite conclusions. Firstly, that the larval development shows that the Sponges cannot be considered Cœlenterates; and, secondly, that the cells forming the connective tissue layer cannot be considered comparable with the mesoblast of the Triploblastica. These views he expressed with remarkable force and lucidity in his article on Porifera in Sir Ray Lankester's 'Treatise on Zoology,' and in his address to the International Congress of Zoology at Cambridge in 1898. It was not, perhaps, an original idea of Minchin's that the Porifera constitute a phylum

of the animal kingdom independently derived from the Choanoflagellata, but it was to his researches, more than to any others, that we are indebted for the final separation of the group from the Cœlenterata on the one hand, and their dissociation from the phyla with three germ layers on the other.

In the course of his post-graduate studies abroad Minchin came under the influence of Bütschli, who was at the time busily engaged in his famous researches on the physical structure of protoplasm. This experience gave him a special interest in the Protozoa, and led to the important series of researches which were published during the second period of his career. In 1894 he gave to English readers an authorised translation of Bütschli's great work on Microscopic Foams and on Protoplasm (A. and C. Black), and nine years later he wrote the article on Sporozoa for Sir Ray Lankester's 'Treatise on Zoology.' These publications established his reputation as a protozoologist with a wide knowledge of the literature of the subject and a marked capacity for dealing with its problems with philosophical insight; but it was not until 1907 that he published, in conjunction with Dr. H. B. Fantham, his first paper of original research in Protozoology, a description of a new genus of Sporozoon—Rhinosporidium—from the septum nasi of man.

*In 1905 Minchin was appointed a member of the Sleeping Sickness Commission of the Royal Society, and was sent out to Uganda to investigate the relation of the tsetse fly (*Glossina palpalis*) to the trypanosome of sleeping sickness (*Trypanosoma gambiense*). He had married Florence Maud Fontain in 1903, and his wife accompanied him on this expedition. He began his work by a study of the anatomy of the fly, and wrote an account of his observations for a communication that was published in the 'Proceedings of the Royal Society' (vol. 76). In this paper he demonstrated the extraordinary skill he possessed in the study of the anatomy of small insects, a skill that was afterwards shown again in his account of the anatomy of the rat flea (*Ceratophyllus fasciatus*), published by the Quekett Microscopical Society in 1915.

Soon after his return from Uganda the opportunity was afforded him of devoting the greater part of his time to research by his appointment as Professor of Protozoology in the Lister Institute. Freed from the cares of teaching elementary students and of administration, he threw himself whole-heartedly into the investigation of the Protozoa of disease, and soon raised himself to the rank of the first of the protozoologists of his time. Continuing the researches he began in Uganda he discovered all the stages of the encystment of *Trypanosoma grayi* in the proctodæum of the fly, and from this was led to the conclusion that the infection of a vertebrate host may in some cases occur not by the inoculative method, but by contamination, a conclusion subsequently confirmed by other members of the Commission by other methods. Having once taken up the fascinating, but complex and

* I am greatly indebted to Dr. H. M. Woodcock of the Lister Institute for material assistance in writing the account of this part of Minchin's career.

difficult, study of the Trypanosomes, he continued the study of this group for the rest of his career. As the outcome of study in the vacations on the Norfolk Broads he published ('Proc. Zool. Soc.,' 1909) an interesting general account of the trypanosomes and trypanoplasms of fresh-water fishes, illustrated by remarkably beautiful figures. In the course of this study he showed how essential it is for minute cytological details to use a wet method of fixation and to stain by some variety of iron-hæmatoxylin. So impressed was Minchin by the necessity for the best possible technique in studying trypanosomes that before undertaking his great research on *Trypanosoma lewisi* he spent some time in comparing the results obtained by many different fixatives and stains. His observations are recorded in a most instructive paper on the cytology of this parasite in relation to technique ('Quart. Journ. Micro. Sci.,' 1909).

He then turned his attention to the investigation of the life cycle of *Trypanosoma lewisi* in the rat flea, and after arduous study, extending over five years, he published, with Dr. J. D. Thomson as joint collaborator, a complete memoir on this subject ('Quart. Journ. Micr. Sci.,' 1915). The great importance of this elaborate piece of work must be recognised by all protozoologists. It is the only complete account of the life-history of any trypanosome in its invertebrate host, and the detailed description of the various stages and their relation to the epithelial cells of the various parts of the alimentary canal of the host that they infest make it a standard work on which the researches of later investigators must be based. It is, perhaps, in this memoir that we see Minchin at his best. On every page we recognise his infinite capacity for taking pains, amounting almost to genius, his skill in technique, his wide knowledge and sound judgment.

But, although Minchin's interest was focussed mainly upon the Trypanosomes from the time he became Professor of Protozoology, he wrote several shorter papers on the Hæmogregarines and parasitic Amœbæ. To the general zoologist, however, his name will be associated principally with his book entitled 'An Introduction to the Study of the Protozoa,' one of the best and most valuable treatises on a single group of animals that has ever been published. In this work he brought together in a concise form a vast amount of information concerning all the groups of Protozoa, but giving in greater detail the results of recent investigations on those that are parasitic. It is a book characteristic of his clear mind, his profound knowledge and powers of acute criticism.

One of the last acts of his busy life was the preparation of his address as President of Section D at the meeting of the British Association in Manchester last September. It was written at a time when he was suffering from the painful and fatal illness to which he succumbed three weeks later. Unable to attend the meeting himself, he invited his friend, Mr. Heron-Allen, to read the address for him, and with his usual care for efficiency marked those parts that he wished to be emphasized and those parts that could be omitted if time were pressing. Those who were present at the meeting will long

remember not only the intrinsic interest and value of the address, but also the impressive and sympathetic manner in which it was delivered by the good friend in whose house he passed some of the last days of his life. In this address Minchin gave us his philosophical standpoint as regards the origin of living organisms on the earth. He believed that the chromatin represents the primary living substance, that in the organisms known as the Chlamydozoa we have the nearest approach to the earliest organisms, that the formation of an envelope or capsule round the primary chromatin particles giving rise to the periplasm or cytoplasm of the cell, the increase in the number and complexity of the chromatin granules, and the formation of a definite nucleus were subsequent processes in the evolutionary cycle. These and many other aspects of Cytology and Protozoology he treated with a masterly hand, and the address is one which for style and evidence of a cultured mind has rarely been excelled in the history of Section D.

S. J. H.

Captain J. W. JENKINSON, M.A., D.Sc.*

John Wilfred Jenkinson was born in 1871, and educated at Bradfield College. In 1890 he came up to Oxford, having obtained a scholarship in classics at Exeter College. His taste for natural history developed early. Already, as a boy at school, he took a keen interest in botany, and several of his finds are mentioned in Druce's 'Flora of Berkshire.' After taking his degree at Oxford in the honour school of Literæ Humaniores, he turned his attention to the study of zoology, and went to work at University College, London, under Prof. Weldon. On his return to Oxford he joined the teaching staff of the Department of Comparative Anatomy in the University Museum, obtained his doctor's degree in Science in 1905, and next year was appointed University Lecturer in Comparative and Experimental Embryology. The excellence of his work in research and in teaching was recognised, and in 1909 his old college, Exeter, elected him to a Research Fellowship. In 1905 he married Constance Stephenson, who survives him.

An ardent patriot, Jenkinson became a member of the Oxford Volunteer Training Corps as soon as the war broke out. Though over age he applied for a commission, and joined the 12th Worcestershire Regiment in January, 1915. He was promoted to a captaincy in April, and left for active service in the Dardanelles on May 10, one of a draft of six officers from his regiment, attached to the 2nd Royal Fusiliers. Hardly had he reached the

* This and other obituary notices of distinguished men of science, not Fellows of the Society, who have fallen in the War, appear in pursuance of a resolution of Council.

eastern field of war when he fell in one of the fierce attacks on the coast of Gallipoli. Thus died in the service of his country a man of indomitable courage, great physical and mental energy, and remarkable powers of endurance. His loss was felt as a painful blow by his many friends and colleagues in Oxford, and as a severe loss to zoological science.

Jenkinson's scientific work was almost entirely devoted to Embryology. His earliest original contribution was "A Reinvestigation of the Early Stages in the Development of the Mouse" ('Quart. Jour. Micro. Sci.,' vol. 43, 1900), an able criticism of the views of Robinson and Assheton concerning the origin of the germ-layers and trophoblast. It was founded on work partly carried out in the laboratory of the famous Dutch embryologist, Prof. Hubrecht, in Utrecht, to which Jenkinson had paid several visits. Soon afterwards he wrote "Observations on the Histology and Physiology of the Placenta of the Mouse" ('Tijdschr. Nederl. Vereenig.,' vol. 7, 1902), and some years later on the placenta in Ungulates ('Proc. Zool. Soc.,' 1906). In a paper "On the Development of the Ear-bones in the Mouse" ('Jour. Anat. and Physiol.,' vol. 45, 1911) he successfully attacked the difficult problem of the first appearance of these structures, and showed that, in the very earliest stages, the stapes is connected with the hyoid arch, and the incus and malleus with the mandibular arch. Quite lately he published (Oxford, 1913) a little text-book on 'Vertebrate Embryology,' giving an excellent account of the germ-cells, the germ-layers, and the placenta.

But Jenkinson soon became more interested in experimental embryology—a comparatively new science little studied in England, and offering an attractive and boundless scope for research. After publishing a detailed description of the maturation and fertilisation processes in the Axolotl ('Quart. Jour. Micro. Sci.,' vol. 48, 1904), he wrote a number of valuable papers on the relation of the symmetry of the egg to the symmetry of the embryo in the frog, on the effect of various salts and other substances on development, and kindred subjects ('Quart. Jour. Micro. Sci.,' 'Biometrika,' 'Brit. Ass. Reports,' 'Archiv f. Entwickelungs-mechanik'). His best known and perhaps most important work is the 'Experimental Embryology' (Oxford, 1909). Although several books on the subject had appeared abroad, this was the first comprehensive text-book published in England on a new and fascinating branch of zoology. It contains a summary and discussion of practically all the results that had been obtained up to the time of its publication. Clear exposition and sound judgment distinguish this work, in which the author finally pronounces against the neo-vitalistic doctrines of Driesch. The appearance of Jenkinson's book stimulated interest in experimental embryology in this country, and the premature death of the author leaves a gap in the ranks of zoological researchers which it will be difficult to fill.

E. S. G.

THOMAS LAUDER BRUNTON, 1844–1916.

On September 16, 1916, in London, this distinguished physician passed away after a long illness, borne with rare fortitude. Although retired from private practice, Brunton was far indeed from retirement in respect of those public causes to which, with the pious tenacity of his race, he devoted much of his life, and a fervour almost religious in its depth and constancy. A short time before his death the present writer had visited Lauder Brunton, and witnessed both the distress under which he laboured and the ingenious methods he had devised for keeping the evil at bay; not in the desire of a mere prolongation of life, though this indeed were no unworthy intention, but in order to cherish the fire of its last embers for those humane ends which he had so ardently at heart. It was therefore with the more admiration that, about three weeks before his death, the writer received from his friend, now silent, a long and important letter covering certain documents and proposals on the subject of physical education, a movement to which, in his later years, Brunton had given no little energy and guidance, one which, especially for the sake of children and young people, he was pressing forward almost with his latest breath. Fortunately, he has worked with comrades and assistants who will not fail to keep his lamp alight, nor let any of his last counsels be forgotten.

Thomas Lauder Brunton was born of pure Scotch lineage at Hiltons Hill, Roxburgh, in 1844, and received his medical training at the University of Edinburgh, where he had a distinguished academic career.

When we look back upon Lauder Brunton's work as a whole we shall recognise its great merits. As with all other observers, this work was not always up to the high level of his best; but his best, whether we regard it as pioneer work or in the more absolute sense of permanent values, was very good. We may divide it into three periods: the pioneer work, the work of his early prime, and the work of later years.

It is scarcely correct to say that pharmacology as an experimental science did not begin till the middle of the nineteenth century, for multitudes of pharmacological experiments, remarkably systematic and precise for their age, were made upon the venoms of animals and the poisons of plants in the times of Attalus III of Pergamon (died B.C. 133), and of Mithridates Eupator (B.C. 63–31), and by none more assiduously than by those kings themselves. However, dismissing these ancient records, but remembering that modern experimental pharmacology could hardly take form before the separation of the active principles of drugs, as of morphin by Sertürner in 1816, and coming down to our own day, we find that the first modern pharmacological laboratory, *i.e.*, the first systematic foundation of pharmacology as an aspect of experimental physiology (including pathology), and on similar methods,

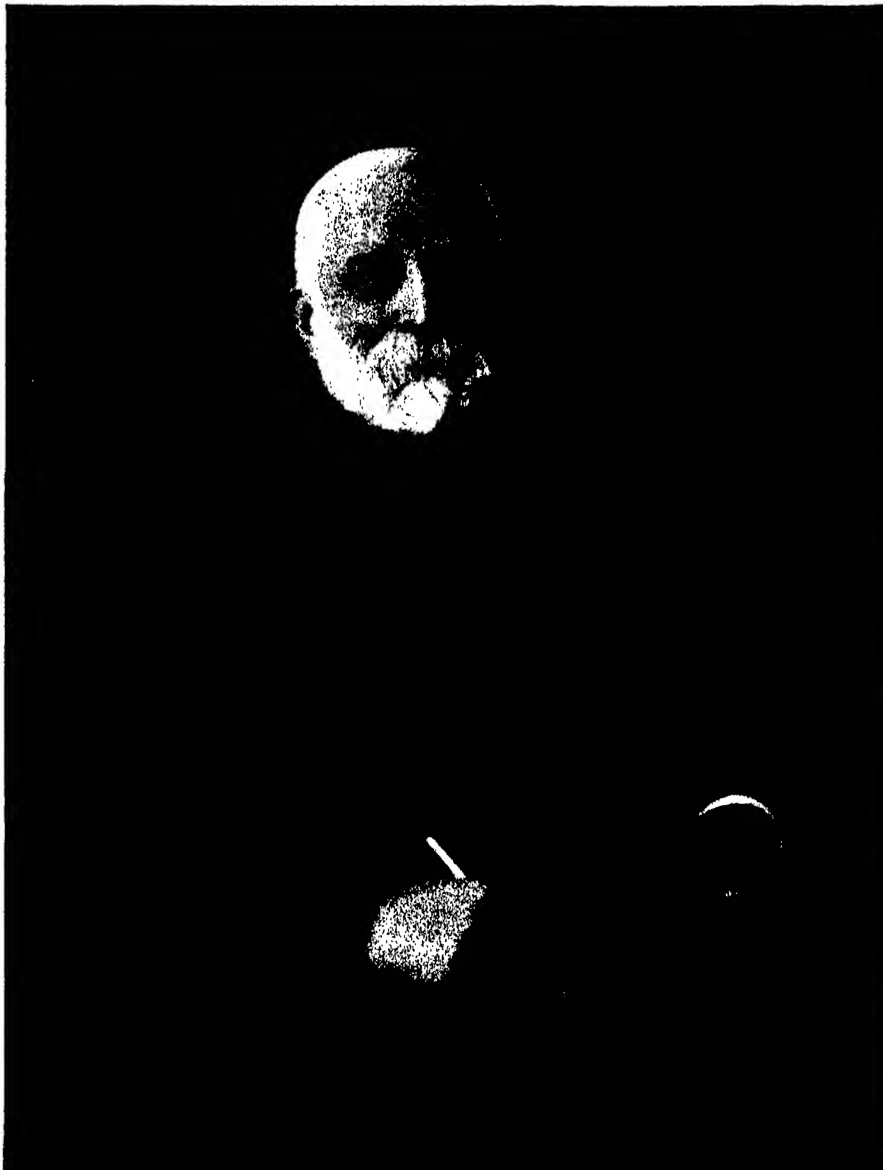


Photo by Elliott & Fry, Ltd.

T. Lauder Brunton

was established by Buchheim at Dorpat about A.D. 1860.* Then quickly followed Buchheim's second foundation at Giessen in 1869; and in 1872 those of Binz in Bonn, of Liebreich in Berlin, of Schmiedeberg in Strassburg. Now what these men did abroad, Brunton and Leech were doing in England. In 1866 the present writer, with his colleague Mr. Teale, was experimenting on animals with morphin, and on the curious tolerance of the drug by rabbits; and in 1868–70 he was studying with Milner Fothergill the effects of digitalis—chiefly on the frog's heart. But without means of maintaining the separated heart in action, and without kymograph and other physiological apparatus, beginners had to content themselves with crude inspection The writer had to relinquish much of the digitalis work to Fothergill, who embodied the results in his Hastings Prize Essay for 1870 (published 1871).

Brunton was appointed Lecturer on Materia Medica at St. Bartholomew's on his 26th birthday, in the year 1871. Leech was appointed (joint) Lecturer on the same subject in Manchester in 1874, Professor in 1881. But Brunton had done good work before he was called to St. Bartholomew's; indeed, in 1871, he had begun to write his 'Experimental Investigation of Medicine.' He was one of the first of the scientific practising physicians who used no empirical remedy without seeking to discover its mode of action, and, by pharmacological and other research, endeavouring to add to our resources. Bence Jones, Golding Bird, Pavy, were of the generation before him, it is true; but few physicians whose interests before all else were, and still remained, clinical, followed scientific investigation so systematically and in so disinterested a spirit. In 1868, after passing through the preliminary stages of his medical education with much distinction, he had presented a thesis for the Degree of M.D., also on the action of digitalis, especially in respect of its effect upon the urine; an essay which recorded a very laborious series of observations upon himself, extending over six months. This thesis was the first-fruits of his many years' labour upon this perplexing drug; yet of all his researches this was, perhaps, the least rewarded. Like many other pharmacologists, Brunton did not sufficiently distinguish between the effects of larger and smaller doses. Perhaps even yet it is not fully realised that the effects of therapeutical doses of drugs cannot always be reckoned as fractions of the effects of poisonous doses, but may be different in kind.

On finding himself, at the conclusion of his hospital course in Edinburgh, the holder of a travelling scholarship, and not without some private means, Brunton decided to devote three years to study in foreign laboratories. First, in 1867, he went to Vienna, and there worked in Brücke and Rosenthal's laboratories; thence to Berlin, still working on digitalis, and deciding that it caused contraction of the peripheral vessels with rise of blood-pressure,† a conclusion now said to be true only of poisonous doses. After a tour in the East we find him, in 1869, in Amsterdam, with Kühne; now engaged upon

* In recent times, perhaps Majendie was the first to work systematically and experimentally with animals on pharmacological problems.—C. A.

† Brunton and Meyer, 'Journ. of Anat. and Physiol.,' vol. 7, p. 135 (1873).

the chemistry of the blood, and of digestion.* Thence he went to Leipsic, and was one of the first students in Carl Ludwig's new institute.† Here it was that he began his admirable researches on the nitrites,‡ and his lifelong friendship with Kronecker.

In 1871 Brunton settled in London and, after a short term at the Middlesex Hospital, was appointed Lecturer on Materia Medica, and Casualty Physician, at St. Bartholomew's. There in a small scullery he began his experimental work in England, some excellent part of it with Fayrer on snake venoms. In this den, and afterwards in the pharmacological laboratory of the new buildings occupied in 1881, he trained pupils and assistants, some of whom afterwards attained professorial rank. As his private practice increased, Brunton was less and less able to work out his problems personally; having laid down the lines of a research he was obliged to leave the elaboration to others. Hence perhaps arose some lack of grip, and a reliance rather upon his highly cultivated intuition than upon patient verification; a bent sometimes noticeable also in his clinical work. When he had formed a vivid idea of a certain process it was sometimes difficult by argument, or even by demonstration, to alter his point of view. But, on the other hand, it was by the aid of this imaginative quality that he did what he did; it was by its light that he saw the new pharmacology, made it for Great Britain, and presented the great gift to his own and to later generations. Moreover it is fair to add that at times, when some absorbing research was on foot, Brunton would leave his large practice, even for weeks, to work with his assistants.

In 1874, at the early age of 30, Brunton was elected Fellow of this Society (Councillor, 1882–4 and 1905–6; Vice-President, 1905–6); and in his earlier days devoted much time and pains to its interests. The Fellowship of the Royal College of Physicians was conferred upon him in 1876: and from other medical and scientific bodies and universities he received many distinctions. While indeed he was still a comparatively unknown man he was, in obedience to the last wishes of Dr. Anstie, appointed Editor of 'The Practitioner.'

In 1885 appeared the first of many editions of the 'Textbook of Pharmacology and Therapeutics': his *magnum opus*, the first complete treatise on physiological pharmacology; it was the summation of 15 years' work and of notes more than once rewritten and of facts re-verified. In lucidity, grasp, and accuracy it stood far before Ringer's book, great as is our debt to Ringer. It contained also a full bibliography, at that time invaluable, for no one so well as Brunton knew what was doing and had been done in his branch of science, and none could so precisely balance the relative values of the results of the several investigators. And meanwhile he was himself

* See Burdon Sanderson's 'Handbook for the Physiol. Laby.,' which contains many useful descriptions by Brunton of experimental method.

† For these dates and places and other records the writer is much indebted to the Obituary Notice in the 'Brit. Med. Jour.,' September 23, 1916; as also for kind assistance to Mrs. Henry, Prof. Bayliss and Prof. Cushny.

‡ 'Journ. Anat. and Physiol.,' 1871, vol. 5, p. 92.

producing excellent papers: on strychnin, caffein, aconite, the nitrites, and so on. In 1867 Brunton made one of the most beneficent discoveries ever achieved in the cure of disease, namely, the relief of angina pectoris by the nitrites (nitrite of amyl: a discovery deserving to rank with that of Peruvian bark in the cure of ague). To have discovered the means of controlling one of the cruellest ills to which man is subject is, perhaps, amid many memorials, that which the author would have prized above all the rewards of his labours.

It is true that his conclusion, that angina is primarily a disease of high blood-pressure, was incorrect, and the sphygmographic tracings on which he based this conclusion showed only that the sphygmograph is not an instrument capable of interpreting pressures, nor even delicate enough to get closely into touch with the finer motions of the pulse. His famous discovery has, therefore, been called a lucky shot, but such hits are made only by persons who have profoundly studied the conditions.

In 1897 Brunton published his 'Lectures on the Action of Medicines,' a work as practical in teaching as delightful to read. As an earnest and kindly physician, in this work, as in his hospital and private practice, he was never drily academic, but kept ever before him the means, empirical as they might be, of bringing medical aid to his patients. If the author were thus disposed to put too strong a faith in drugs as remedies, we should be the last to blame him for so generous an enthusiasm.

This work was followed by the 'Disorders of Assimilation' (1901) and the 'Therapeutics of the Circulation.' In 1885 he had delivered the Lettsomian Lectures on 'Disorders of the Digestion,' published in 1886. As in pharmacology, so in nutrition, secretion, and excretion, Brunton was a pioneer. He set on foot much of the recent work on ferments and enzymes, on processes of digestion and assimilation, and even on the organotherapy and immunity investigations which are now in the forefront of research. Concerning this part of his work, reference may be made to his Address to the International Congress of Medicine at Moscow in 1897. But to enumerate all Brunton's contributions to the science and art of Medicine would be a long task. Versed in many subjects of clinical and therapeutical interest, and a prodigious worker, his books, reports, and essays were not only numerous, but were republished and re-embodied in many shapes. They were principally concerned with gastric and cardiac problems, for the most part of much practical value; and, in later life, with public health, such, for example, as the effects of exercise and stress on the heart, the need of physical education, of public hygiene, and so forth. He served also on the Commission of 1886, which reported on Pasteur's treatment of hydrophobia.

In the episode in his career known as the Hyderabad Commission (1889) Brunton was less successful. The whole affair was too brief, too hurried. It is true that in chloroform poisoning the respiration is affected, but this is a subsidiary hazard, and one readily countered by artificial respiration. The effect upon the heart is the chief peril, as was finally proved in the Cambridge Physiological Laboratory, in the presence of Lieut.-Colonel Lawrie

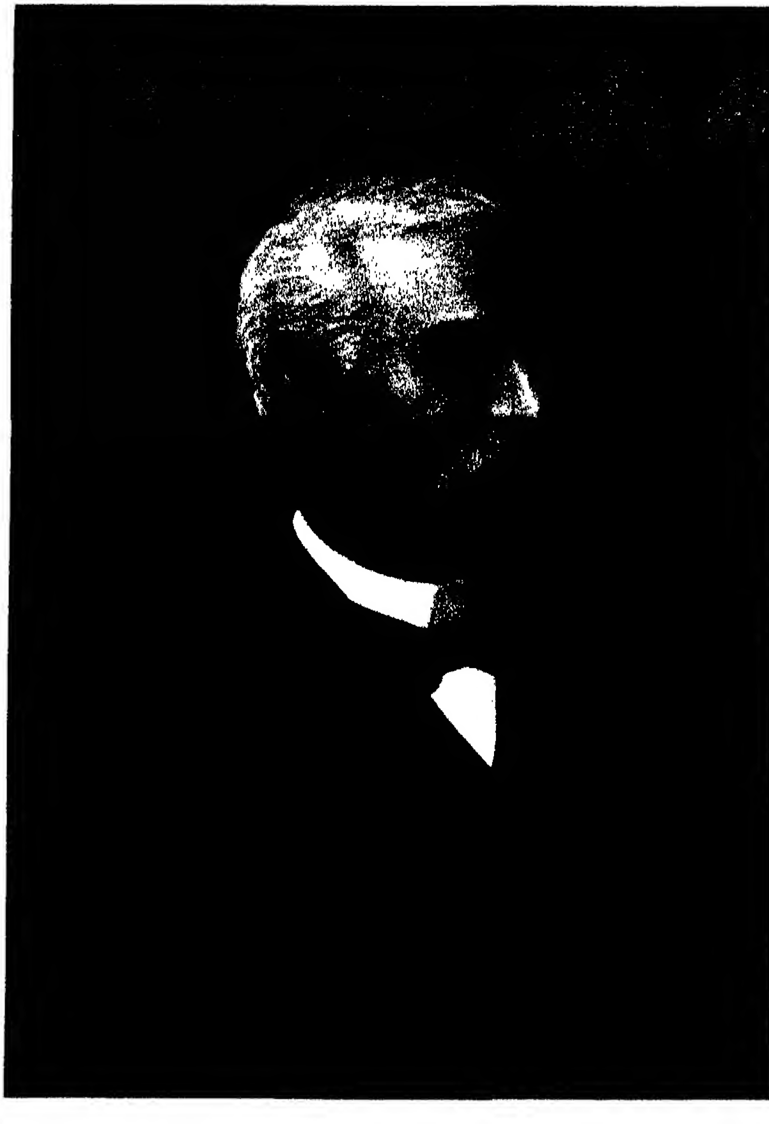
and his assistants by the cross-circulation experiment devised by Dr. Gaskell and performed by Dr. Shore; a demonstration in which the present writer took a subordinate part. Nevertheless, if the results in some respects were doubtful, and in others needed re-interpretation, the whole problem was raised to the plane of its eminent importance.

It appears that, for some years past, from his intimate knowledge of the German people, Brunton had foreseen their propensity, sooner or later, to force a war upon this country. He feared, however, that we should never submit to conscription, unless possibly in a crisis such as the present. For this reason, and in furtherance of his scientific work on hygiene and dietetics, he founded the National League for Physical Education and Improvement, an organisation devoted to the nurture, from infancy upwards, of a healthy, vigorous, and high-spirited people. The League, it is to be hoped, is now so far established in the public favour and interest as to survive the loss of its leader. The fulfilment of its purpose would be his most signal memorial.

By academic and official decorations Lauder Brunton was richly distinguished—he was knighted in 1900 and received a baronetcy in 1909; but perhaps, in his loyal and patriotic heart, the honour of none of these was to be compared with that of the devotion of his younger son, a promising Cambridge medical graduate, who last year gave his life on the field of battle for his country; his "dearest pride," as he sought to make it, but also a bereavement which, falling but a brief five years after Lady Brunton's death, deepened the shadows of his latter days. Happily his elder son, also on military service, and his devoted daughters were still spared to him.

Brunton was happy both in his life and in his death, comforted in his passing away by the respect and affection of all who knew him. Indeed, no one could speak with him without being affected by his sweet, persuasive enthusiasm, his keen intelligence, his gentle acceptance of all differences of opinion, his frank, affectionate courtesy, and that faith in his art and in mankind, which engendered a like faith and hope in those who only too often sorely needed these consolations.

C. A.



Hugo Kronecker.

HUGO KRONECKER, 1839–1914.

Hugo Kronecker was born on January 27, 1839, at Liegnitz, in Prussia. His father was a merchant; his elder brother, Leopold, the well-known mathematician. Hugo studied Medicine at Berlin and at Heidelberg; at the latter place he came under the influence of Helmholtz, and commenced an enduring friendship with Kuehne. His graduation thesis was on muscular fatigue, a subject which remained of permanent interest to him.

Kronecker's original intention was to devote himself to clinical medicine; accordingly, in 1865, he accepted an Assistantship under Traube in Berlin. But owing to a threatening of pulmonary disease he went to live for a time in Pisa; here he acquired a mastery of the Italian language and an affection for things Italian which lasted throughout life. He was also an accomplished French scholar, but although he knew English well he was always somewhat shy of speaking it.

The turning-point in his life came in 1868, when he started working at the famous Institute of Physiology at Leipzig, where he became Assistant in 1871, and Extraordinarius in 1874. In 1876 he was invited by du Bois-Reymond to undertake the direction of Experimental Physiology in the Berlin Laboratory, a position which he held until 1884, when he was appointed to the Chair of Physiology at Berne. In Berne he completely re-organised the department, and a new Physiological Institute, which he termed the "Hallerianum," after the famous old Berne physiologist, Albrecht von Haller, was erected under his auspices. Within the walls of this Institute, and in a modest but delightful dwelling which he built for himself close by, he passed the remainder of his life, happy in his work and in the visits of his friends, to whom he loved to dispense a cordial hospitality; happy especially in the companionship of his charming wife and family, which consisted of a son and daughter.

It was in Leipzig, in 1876 (during the course of a summer semester spent in Ludwig's laboratory), that the writer first came to know Kronecker; the acquaintance there formed grew into an intimate friendship. At that period the Institute was in full activity; a large number of investigators, gathered from all parts of the world, were working there. Of those who were present at the same time as the writer, the names of von Basch, Baxt, Bowditch, Buchner, Cyon, Drechsel, Flechsig, von Frey, Gaskell, Gaule, Hesse, Merunovicz, Mosso, and Schwalbe, spring at once into one's memory and serve to gauge the scientific activity of the community which centred around the genial master, Carl Ludwig, who personally directed all the work of the Institute. But of its social activity Kronecker was the leader, and much of the pleasure which those who were working in Leipzig experienced was due to the tactful manner in which he contrived to bring

together into happy intercourse the heterogeneous elements of which the little group of fellow-workers consisted.

Kronecker can hardly be described as a great physiologist. His work, although abundant and sound, is in no way epoch-making. He was meticulously accurate to the smallest detail; this rendered the progress of his investigations slow, but it also rendered it sure. His favourite subject was the nature of cardiac action. It was he who first elaborated a practical method for recording the contractions of the isolated frog-heart which enabled the "staircase" phenomenon and the "all or none" condition to be satisfactorily investigated. Later he turned his attention to the mammalian heart, in which he endeavoured to demonstrate the existence of a peripheral nervous centre. Himself a keen alpinist, he took an active interest in the organization of the stations established in the High Alps by Italian physiologists, and especially by his friend Angelo Mosso, for the purpose of recording observations on the effects of life at high altitudes upon the bodily functions. And in collaboration with his pupil Meltzer, he added materially to our knowledge of the mechanism of deglutition; the result of the joint investigation being to demonstrate for the first time the method by which a fluid bolus is carried from the mouth to the stomach.

The devising of new methods and the designing of new forms of apparatus was ever a source of pleasure to Kronecker. It is to him we owe the only precise method of graduation of the sliding induction coil which we possess, as well as many new and ingenious devices in other apparatus. His partiality for this kind of work is evidenced by the interest he took in the establishment of the Marey Institute in Paris, which was formed for the demonstration of new physiological methods, and it says much for the universal appreciation in which Kronecker was personally held that, although a German, nay, even a Prussian, he was for several years President of that essentially French institution.

It was with the active assistance and collaboration of Kronecker that the series of International Congresses of Physiology, which have proved so successful, was established, although the chief credit for them must be given to Sir Michael Foster and the Physiological Society of Great Britain. It will be many years before such a Congress can meet again. And no one would, we may be sure, have regretted more than Kronecker the estrangement with so many of his friends which the war must necessarily have produced. But he died, suddenly, at Nauheim, two months previous to that fateful August of 1914; and if we consider the effect which would have been produced upon his affectionate nature by such estrangement, his friends in this country, and they were many, much as they deplore his loss, cannot but think regarding him, *Felix opportunitate mortis!*

E. A. S.

ELIAS MECZNIKOW, 1845-1916.

One of the most remarkable figures in the scientific world passed from among us on July 15, 1916. Elie Metchnikoff, as they wrote his name in France, his adopted home, stands out as the type of a gifted, indefatigable investigator of Nature who, in accordance with his beautiful and earnest character, never faltered in his career, but from his boyhood onwards devoted himself to the minute study of animal life. By a natural and, as it seemed, inevitable process he passed through the study of the microscopic structure and embryonic growth of simple marine organisms to the investigation of human diseases and to his great discoveries of the nature of the process known as inflammation and of the mechanism of "immunity" to infective germs and the poisons produced by them. By every zoologist in the world he was especially honoured and revered; for it was to him that we owed the demonstration of the unity of biological science and the brilliant proof of the invaluable importance to humanity of that delightful pursuit of the structure and laws of growth and form of the lower animals which he and we had pursued from pure love of the beauty and wonder of the intricate problems of organic morphology.

Just as his chief and friend, the great Pasteur, was privileged to proceed directly and logically in his own life's work, by his genius and insight, from the discovery of astonishing new facts as to crystalline structure—which seemed to have no bearing on human affairs—to the understanding (by the aid of those discoveries) of fermentation and infective disease; so did Metchnikoff himself both discover the activity and universality of the organic cell-units which he called "phagocytes," and at once proceed to give the demonstration of their prime importance in the process known as inflammation and the understanding of "immunity," which has revolutionised medical theory and practice.

Metchnikoff, though one of the greatest benefactors of mankind, by his discoveries as to the nature and consequent treatment of disease, did not set out on his career with any preconceived intention of arriving at what are called "practical results." Like so many others of the greatest discoverers, he was attracted to the field of his life's work by a delight in its beauty. His æsthetic sense was gratified by the observation and discovery of the relations of the marvellous phenomena of structure and function in the teeming life of earth and sea—only revealed by the microscope, and then only to the strenuous, conscientious, and faithful student. He wandered, as though led by instinct, into this enchanting "fairyland," and, as has happened to others, in the midst of his ever-varied, ever-fascinating experiences, he suddenly and unexpectedly found himself in sight of something different—a thing of vast and immediate value to humanity. He saw under his hand, as it were, a discovery which might prove to be of

enormous importance to the life and health—"the well-being"—of mankind. He had become familiar with the food-ingesting amœba-like cells in the blood and tissues of minute transparent animals—so minute and so transparent that he could watch them in life, with a powerful microscope, without damaging or interfering with their activities. He saw, in a flash of prophetic insight, the significance of these "eater-cells," or "phagocytes," as he named them. His knowledge and training in the sciences of chemistry, physiology, anatomy, and pathology enabled him to guess correctly that it was their business to engulf and destroy noxious intrusive particles which might find their way into the animal of which they formed part, and, above all, to attack and destroy those agents of infective disease, the bacteria and other microbes, which had been but recently recognised, owing, in the first place, to the work of Pasteur, as the specific causes of a whole series of deadly maladies. Thus his pleasant tentative explorations in microscopic zoology were arrested. He had found a great treasure. It was not one which would enrich him—for that he had no ambition. But it would, he foresaw, in all probability prove to be of vast service to his fellow-men. It became his duty, and no less his pleasure, to devote himself to the securing and safe delivery of this great treasure, and all that it carried with it, to the world of men. In this spirit, and with splendid success, he devoted the rest of his life, untiringly, to the foundation and elaboration of his doctrine of phagocytosis.

Elie Metchnikoff was born in 1845 at Ivanavka, near Kharkoff. His father was of Moldavian ancestry and an officer of the Imperial Guard, from which he retired with the rank of major-general. He was devoted to the pursuits of a country gentleman, among which horse-racing was his special favourite. He had no tendencies to scientific study. Elie's mother, whose family name was Nevakovitch, was a Jewess. He owed his mental gifts largely to her. From childhood he showed a strong taste for the study of Nature. After passing through the high school of Kharkoff he entered the university at the age of 17 and completed his degree examinations in two years, when he went off (in 1864) to Germany for further biological training.

He had already, in 1863, when he was only 18, published a paper in Reichert's 'Archiv' on the stalk of Vorticella, and another on the nematode Diplogaster. In 1864 he published some observations on the Acinetarian Sphærophrya. After a brief sojourn in Heligoland he went to work in Leuckart's laboratory at Giessen, and accompanied the professor when the latter was promoted to the Chair of Zoology in the University of Göttingen. In Leuckart's laboratory he worked at the parasite of the frog, *Ascaris nigrovenosa*, and made the important discovery of the fact that the hermaphrodite parasite of the frog's lung hatched from eggs gives birth viviparously to a free-living generation of males and females. This he published in 1865 in Reichert's 'Archiv,' and a translation of his paper appeared in the 'Quarterly Journal of Microscopical Science' in 1866. Leuckart claimed to have made the discovery "with the assistance of

Herr Mecznikow," but Metchnikoff briefly stated that this was erroneous and that he alone had done the work, in the absence of Prof. Leuckart and without his aid or suggestion. Naturally this terminated their friendly relations. In the same year he published some notes on those little-known microscopic animals, Icthydium, Chætonotus, Echinoderes, and Desmoscolex. This also was translated for the 'Quarterly Journal' in 1866, and thus I became familiar with his name and the interesting character of his work, though I did not make his personal acquaintance until 22 years latter, when (in 1888) Pasteur introduced me to him in his laboratory in the Rue Vaugirard.

These papers were rapidly followed in 1866 by others showing his first-rate powers of accurate observation and originality, viz. on a European land Planarian; on the development of Myzostomum, the ecto-parasite of the feather-star, which he showed to be a modified Chætopod; on insect embryology (Hemiptera and Diptera); on the remarkable new rotifer, *Apsilus lentiformis;* and on the viviparous reproduction of the larvæ of the fly Cecidomyia. Then he sojourned for a time (1867) at Naples (before the days of Dohrn's Zoological Station) and wrote on the embryology of the cuttle-fish Sepiola, on the strange marine forms Chætosoma and Rhabdogaster, and in 1869 on Tornaria (which he showed to be the larva of Balanoglossus) and on the embryology of Echinoderms and of jelly-fish.

In 1870 he was appointed Professor Ordinarius of Zoology in the University of Odessa, and soon afterwards published papers on the embryology of Chelifer and of Myriapods. In the previous year he published an interesting paper on the little Trematode parasite of fishes' gills—Gyrodactylus—and joined with that fine naturalist, Claparède, whom he met at Naples, in a paper on the embryology of Chætopods.

After his appointment at Odessa his work was interrupted by the illness and death from tuberculosis of his first wife, whom he had married in 1868. In spite of every care and a long sojourn in Madeira, whither he accompanied her, she died there in 1873.

In 1874 we find a paper by him "On the Eyelids of Mongolians and Caucasians," of considerable value to anthropologists, and in 1877 one of a bionomic character on "The Struggle for Existence between Two Species of Cockroaches—*Periplaneta orientalis* and *Blatta germanica.*"

In 1875 he married his second wife, Olga Belocoyitoff, who was only 17 years of age. She had just completed her studies in the "lycée" of Odessa, and attended after her marriage her husband's zoological teaching in the university. She survives him, and was, for 40 years, his constant companion and ceaselessly devoted friend and helpmeet. She often aided him in laboratory work and by her knowledge of English and other languages, though her special gifts, which she has cultivated to a high degree of excellence, are in painting and sculpture. From time to time she has published her own contributions to subjects which were occupying her husband's attention. The earliest of these is one "On the Morphology of the Pelvis and Shoulder-

girdle of the Cartilaginous Fishes," published in the 'Zeitsch. Wiss. Zoologie,' 1880.

Metchnikoff holds an important place beside his great fellow-countryman and intimate friend, Alexander Kowalewsky (who died some years ago), in the establishment of what may be called cellular embryology and the investigation of the early stages of development of invertebrata by following out the process of cell-division and the arrangement of the early formed cells in layers. In the 12 years 1875 to 1886, when his last embryological paper was published, he produced many important memoirs on cellular embryology—namely, on that of calcareous sponges (in which he showed that the inner and outer primitive layers had been transposed in regard to their origin by Haeckel and Miklucko-Macleay); on that of jelly-fishes, of Planarians, of Echinoderms, of Ctenophora, and of Medusæ. These were accompanied by important theoretical discussions and suggestions as to the ultimate ancestral origin of the endoderm and the mesoblast. He also wrote on that curious group of minute parasites, the Orthonectids, and on insect diseases.

But the new departure in his fruitful career was approaching. It grew out of his observations on living jelly-fishes and sponges and on the transparent marine embryos of Echinoderms and the transparent floating mollusc Phyllirhöe. In 1882, owing to political disturbances in the University of Odessa, Metchnikoff migrated to Messina, the harbour of which is celebrated among zoologists for its rich fauna of transparent floating larvæ and adult glass-like Pteropods and jelly-fishes. Here he developed his views, already foreshadowed in 1880 ('Zoolog. Anzeiger'), on intracellular digestion exhibited by the amœboid cells of animal organisms, and published a series of papers in which the name "phagocyte" is first applied to these cells. In this, as in similar cases of discovery, neither Metchnikoff himself nor any of his friends claimed that he was the first to observe all the facts leading to his generalisation. He was *not* the first to witness the ingestion of foreign particles, of fragments of dead tissue, and even of bacteria, by the amœba-like cells of the animal body. He knew and cited the early observations of Haeckel on the ingestion of pigment granules by the amœboid blood-corpuscles of the sea-slug Tethys. He knew and cited the numerous observations on the activity of large amœboid cells in assisting the resorption or rapid destruction of other tissues in some special instances. He knew the observations of Jeffrey Parker and others on the intracellular digestion of food particles taken into their substance by the endoderm cells lining the digestive cavity of Hydra. He knew Koch's observation of bacilli within a colourless vertebrate blood-corpuscle, attributed by that observer to the active penetration of the blood-corpuscle by the aggressive bacilli. These and other like instances were all regarded as exceptional by their observers and not interpreted as evidences of a definite and universal activity of the amœboid cells of large physiological significance. Metchnikoff was acquainted with the remarkable discoveries of Cohnheim, Stricker, and others (in some of which I had a pupil's share during my stay in the winters of 1869–70 and 1870–71 at Vienna and Leipzig

respectively). The pathological laboratories were full of observations and talk about the "diapedesis" and "out-wandering" of the amœboid corpuscles in inflamed tissues, the origin of pus-corpuscles, and the activity of the amœboid cells in the stellate cavities of the frog's cornea and other connective tissues when stimulated. Metchnikoff put two and two together, and formulated the proposition that in all multicellular animals the main function of the cells derived from the deep or mid-embryonic layer between the dermal and intestinal lining layers is (with exception of those which become "muscular cells") nutritional, and that they possess the power of ingesting and digesting —as does an amœba—solid particles, whether such particles are introduced from the outside or are parts of the organism which, owing to one reason or another, must be broken up and removed. The amœboid cells in connective tissues and in the blood and lymph are such eater-cells or phagocytes, as he now termed them.

He at once proceeded to explain the significance of these phagocytes and their utility to the organism, not only by pointing to their work as scavengers removing injured and dead tissue, to which they are brought in hundreds of thousands by the process known as inflammation, but he also immediately gave first-class importance to their recognition by connecting them with Pasteur's great discoveries as to the cause of infective diseases by poisonous "microbes" which intrude into previously healthy organisms. He further connected his generalisation with Darwin's theory of the origin of species by the natural selection of favoured races in the struggle for existence. He published in 1884 an essay entitled 'The Struggle of the Organism against Microbes,' in which he maintained the thesis that the phagocytes, universally present in multicellular animals, have been developed and established by natural selection in the animal organism as a protection against intrusive disease-causing bacteria.

He was able in 1884 to observe and give illustrative drawings of a demonstrative case of the activity of the phagocytes in the blood of a transparent fresh-water flea (Daphnia) when it was infected by a yeast-like parasite called Monospora. This parasite frequently makes its way into the blood of the water flea and, multiplying there, often causes death. Metchnikoff watched with his microscope and made careful drawings of the phagocytes as he saw them in the living flea engulfing and digesting the intrusive Monospora. In some cases the phagocytes, in others the Monospora, got the upper hand. Later, when I knew him, he had a small aquarium dedicated to the cultivation of these demonstrative water fleas and their infective microbe.

Having now determined to give up his zoological and embryological researches in order to devote the rest of his life to the development of his doctrine of "phagocytosis," Metchnikoff accepted the invitation to become director of a new bacteriological laboratory at Odessa, but, finding the conditions there not favourable to his special work, he relinquished the post in 1888 and, having fortunately been cold-shouldered in Berlin, came to

Pasteur in Paris, who, thoroughly appreciating the value of his work, gave him a laboratory and every facility for his investigations in his own institute, at that time located in the École Normale, rue Vaugirard. When, a few years later, the Institut Pasteur was built in the rue Dutot, Metchnikoff was given a fine suite of laboratories, lecture-room, and space for keeping animals, and became sub-director of the Institute a few years ago.

Young investigators now came in growing numbers to Paris in order to work in Metchnikoff's laboratory, and he pursued with triumphant success, but not without opposition and sometimes insult from the older and more ignorant medical men, the establishment of his views as to the essential importance of "phagocytosis" in resistance to disease. Among his more fatuous opponents was a prominent English pathologist who scornfully alluded to his views as "Metchnikoffism."

In 1892 he produced as an illustrated volume, with the title 'The Comparative Pathology of Inflammation,' the substance of a course of lectures delivered at the Institut Pasteur. It is one of the most delightful examples of scientific method conceivable. It is essentially a careful and logical presentation of minute observations arranged so as to bring before the reader the evidence in favour of his argument. He invariably followed this method in the controversies in which he necessarily engaged. He never recriminated; he never cited mere authority nor endeavoured to falsify his opponent's statements by "smart" word-play. He simply made new experiments and observations suggested by his adversary's line of attack, and so practically smothered him by the weight of honest, straightforward demonstration of fact. He showed that in the lower animals the phagocytes are attracted in hundreds by "chemiotaxis" to intrusive or injurious bodies which occur in the tissues, and then either enclose or digest them. He proceeded to show that in the vertebrates, where the immense network of the blood-vessels is under the control of the nervous system, "inflammation" is set up as a curative process, and that the elaboration of its mechanism has been established by natural selection. A local arrest of the blood-stream is produced by the nerve-control of the vascular system, resulting in the out-wandering from the now nearly stagnant blood of phagocytes chemically attracted to an injured spot, where, arriving like an innumerable crowd or army of scavengers, they proceed to engulf and digest tissue which has been killed by injury, and similarly to isolate or to destroy and digest injurious intrusive substances, prominent among which are infective poisonous bacteria.

Metchnikoff thus finally and conclusively "explained" the process called "inflammation." His attention, and that of his pupils, was now given for some years to the great question of "immunity." How is it that some individuals are either free from the attacks of parasitic micro-organisms to which their fellows are liable, or, if attacked, suffer less seriously than others do? To answer this question is to go a long way to the solution of the great practical question as to how to produce immunity to infective disease in man. It involved the investigation of the chemical activities of the phago-

cytes, to the knowledge and theoretical understanding of which a great number of highly gifted leaders of experimental inquiry—to name only Ehrlich, Behring, and Almroth Wright—have contributed in the most important way. A large series of investigations and records of experiment was now continuously produced by Metchnikoff or by his assistants under his immediate supervision. The 'Annales de l'Institut Pasteur' are largely made up of these records and discussions. In 1901, Metchnikoff produced his great book on 'Immunity in Infectious Diseases,' an English translation of which was at once published. The subject branched out into various lines, such as are indicated by the names serotherapy, toxins and anti-toxins, hæmolysis, opsonins, and bacteriotropins. It must suffice here to state that Metchnikoff successfully established the doctrine that it is to the healthy activity of our phagocytes that we have to look not only for temporary protection, but for immunity against the micro-organisms of disease.

Since 1901—until he fell ill last winter—Metchnikoff was incessantly active in his laboratory, working there from early morning until evening, when he took train to his country house on the heights above the Seine. Rarely would he tear himself away from his absorbing work to enjoy a holiday. He went a few years ago to Astrachan, on the Caspian, to enquire for the Russian Government into the occurrence of bubonic plague in that region, and studied also the incidence of tuberculosis in the town populations and among the Kalmuck Tartars. On the latter subject he gave (in response to my urgent request) a valuable lecture in London before the National Health Society (in 1912), and on other occasions he made short visits to this country in order to receive honours and deliver special discourses—as at the Darwin Celebration at Cambridge in 1909. The variety of infective diseases to the experimental investigation of which he turned the resources of his laboratory and his theoretical conceptions is truly astonishing. As late as 1911 he wrote: "Perhaps before long it will be possible to explain diabetes, gout, and rheumatism by the injurious activity of some variety of microbe" (preface to the excellent volume, 'Microbes and Toxins,' by Dr. Etienne Burnet, published in London by Heinemann).

In 1903 he found time to write a profoundly interesting popular book, 'The Nature of Man' (London: Heinemann), in which, among other things, he discourses of old age, and his view that unhealthy fermentation commonly occurring in the large intestine produces poisons which are absorbed, and lead to deterioration of the tissues of the walls of the arteries, and so to senile changes and unduly early death. He satisfied himself, experimentally and clinically, that the use of "sour milk" as an article of diet checks or altogether arrests this unhealthy fermentation in the intestine by planting there the lactic bacillus, which, forming lactic acid, renders the life and growth of the bacteria of those special poisonous fermentations (which cannot flourish in an acid environment) impossible. Hence, he himself daily took a pint or so of sour milk, and he recommended it to others, and arranged for the commercial preparation of a particularly pure and agreeable

"sour milk," from the sale of which he scrupulously abstained from deriving any pecuniary profit. This small, though valuable, adventure of his in dietetics has been—unfortunately, but perhaps inevitably—the one and only feature of his long career of vast scientific discovery which has impressed itself on the somewhat erratic intelligence of the "man in the street."

In this connection, Metchnikoff made many investigations as to the presence of various kinds of bacteria in the intestines of both vertebrate and invertebrate animals, and the importance of those parasites in the digestive process. Investigations were carried on in his laboratory as to the possibility of keeping the digestive canal of new-born animals (tadpoles, chicks, rabbits, and insect larvæ) free from bacteria and the result upon the digestive process. He found that the digestive canal of the large frugivorous bats was very nearly free from bacterial infection, and he spent some time himself, and employed one of his assistants, in studying in London the condition, in regard to this matter, of patients from whom Mr. Arbuthnot Lane had removed the large intestine. His work on this subject was still in progress.

Another piece of work of immense importance to the health of the community, which we owe entirely to Metchnikoff, is the demonstration, carried out, in the first place, by experiments in his laboratory on Chimpanzees, and later confirmed by the voluntary submission to experiment on the part of a young French medical man, that the application of calomel ointment is a definite preventive of the transference of the syphilitic virus from one individual to another. This discovery has been made widely known, and has been officially and systematically applied by the public medical authorities in France, Germany, Austria, and other countries, with the most satisfactory results. Metchnikoff himself anticipated that, in a few years' time, the knowledge of this simple preventive would entirely extirpate the terrible disease in those countries sufficiently civilised to make use of it.

Metchnikoff was a Foreign Member and Copley Medallist of the Royal Society, a Member of the Institute of France, of the Academy of Sciences of Petrograd, and of many other societies. In 1908 he was awarded the Nobel Prize for his researches on immunity, and he received only a fortnight before his death the announcement that the Albert Medal of the Society of Arts of London had been awarded to him in view of the benefit to humanity of his scientific discoveries.

I cannot close this imperfect survey of the impressive and ideally complete career of my friend without some few personal notes. From the day when I met him in Pasteur's laboratory in 1888 we became warm friends. He was singularly simple, genuine, and unaffectedly good and unselfish. I could tell a hundred tales of his benevolence and humane spirit; of the unrecorded charitable aid given by him and his wife to the poor of Paris and to expatriated Russians; of his genuine kindness and consideration for all those who were his servants. I am convinced that the devotion of the latter half of his life to the solution of the problems of disease was due to his goodness

of heart and his ardent desire to alleviate human suffering. He never was a smoker, and 20 years ago gave up the use of alcohol entirely. He had no taste for sport of any kind, and never indulged in "recreations" or "amusements" or big social functions. He was a devoted lover of music, and had much knowledge of art and many friends in the great art world of Paris. His beard was large and his hair long, and he was thick-set and muscularly strong, though he became more and more bent, as the years went on, by his constant stooping over the microscope. No year passed, after I first knew him, without my spending some time with him and Madame Metchnikoff in Paris or in their home at Sèvres, and on several occasions he has stayed with me in London or earlier in Oxford. From time to time he has shown to me the experiments and microscopic evidence upon which his own and his pupils' discoveries were based, and has put before me the preliminary hypotheses by aid of which he was seeking—as opportunity offered—to arrive at further knowledge of appendicitis, syphilis, the yaws, infantile paralysis, green diarrhœa, cholera, tubercle, cancer, diabetes, gout, and rheumatism. Only three years ago he carried out some new researches on a zoological subject—the natural removal of black pigment from the wing-feathers of gulls—which he proposed to publish in the 'Quarterly Journal of Microscopical Science.' But the terrible events of the last two years put such work out of his power. In his last moments he insisted very urgently that an immediate autopsy should follow his death. He had suffered for six months from pneumonia, pleurisy, and latterly bronchitis. The autopsy showed atheroma of the aorta and related cardiac disease. Metchnikoff died in the apartments of the Institut which had been assigned as a dwelling to Pasteur. According to his wish, his remains have been incinerated, and the urn containing his ashes is placed in the library of the Pasteur Institute.

E. R. L.

[The preceding article is a corrected reprint (with some additions) of that published by the writer in 'Nature,' July 27, 1916.]

HAROLD HENRY WELCH PEARSON, 1870–1916.

THE death of Prof. Pearson is not only a calamity from the point of view of the welfare of South African Botany, both pure and applied, but it also means the removal from the ranks of botanical investigators of an exceptionally active worker and an original thinker, who combined in an unusual degree the power of doing work with an intellectual equipment and temperament which enabled him to do it well and thoroughly. Few men have made better use of their opportunities or have done as much as he did to advance botanical science in so short a period. It is not only as a botanist that Pearson will be missed: he had many friends both in South Africa and in England who appreciated his singularly attractive personality and felt for him a deep affection. His wide interests, his passion for grappling with botanical problems, and his love of Nature made him an inspiring teacher and a successful lecturer. In South Africa he had friends throughout the Union, men of all shades of political opinion and social standing; his honesty of purpose, his geniality, and devotion to duty enabled him to win the affection of those with whom he was associated, and to exert a wide influence not merely in scientific matters but in the affairs of daily life.

Harold Henry Welch Pearson was born at Long Sutton, in Lincolnshire, on January 28, 1870, and died on November 3, 1916, at the Mount Royal Hospital, Wynberg, Cape Town. After recovering from the effects of an operation he contracted pneumonia, which was the actual cause of death. He was privately educated; after holding a teaching post at Eastbourne he entered the University as a non-collegiate student in 1893. In October, 1896, he became a member of Christ's College, and in 1898, in consequence of his election to a Frank Smart Studentship, he migrated to Gonville and Caius College. His career at the University was a series of successes. As an undergraduate he was a particularly alert student; he had a keen sense of humour and thoroughly enjoyed the best side of Cambridge life. As he rapidly developed both intellectually and in his knowledge of men, he retained his boyish enthusiasm and a disposition unspoilt by closer contact with the world.

Pearson was appointed Assistant Curator of the Cambridge Herbarium in 1898, and in 1899 he became a member of the staff of the Herbarium of the Royal Gardens, Kew. In 1903 he entered upon his duties as Harry Bolus Professor of Botany at the South African College, Cape Town. He was the first occupant of the Chair, and it would be difficult to find a man as well qualified as he proved himself to be to set a standard for succeeding generations. Before leaving England he married the youngest daughter of the late William Pratt, of Little Bradley, Suffolk; his widow alone survives

him. In 1916 he was elected a Fellow of the Royal Society. On hearing of his election, he wrote :—" I was a little surprised to find myself in the list this year; that I was pleased needs no statement. Under any circumstances I should appreciate the honour immensely; under my particular circumstances I think I value it more than I should have done under some others—isolated here from the centre of the things that interest me, this distinction means a very great deal to me."

Pearson's first paper, published in the 'Annals of Botany' in 1898, deals with the anatomy of seedlings of the Queensland Cycad *Bowenia spectabilis* and includes a description of the apogeotropic roots of both young and adult plants. Though he did not publish much on the Cycadaceæ, Pearson made several important additions to our knowledge of this exceptionally interesting family, notably through his own observations in the field and the more continuous records kept at his suggestion by residents in the Cycad country. He obtained convincing evidence of entomophily in *Encephalartos villosus*, and in many other directions added to the meagre information available with regard to the ecology and natural history of African Cycads. He always had a special affection for Cycads, and was justly proud of the splendid collection of living plants cultivated under his supervision in the Kirstenbosch Garden. In 1897, aided by a grant from the University Worts Travelling Fund, Pearson left Cambridge for Ceylon. The comparatively short time spent in the island was mainly devoted to the investigation of the Patanas, a peculiar savannah-like vegetation in the central mountain group at approximately all altitudes over 2000 feet. At the suggestion of Dr. J. C. Willis, then Director of the Peradeniya Gardens, Pearson undertook the task of discovering the causes which led to the development of the patanas, and of ascertaining to what extent the vegetation showed adaptation to the ecological factors under the influence of which it had been selected. He came to the conclusion that the peculiarities of the climate have co-operated with periodically recurrent grass-fires in transforming an open forest into barren grassy plains; he made observations on the biological features of the flora, and discussed the factors concerned with the production of both wet and dry patanas, that is the vegetation above and below 4500 feet. In the investigation of the anatomy of the vegetative organs he obtained the assistance of his friend Mr. J. Parkin. It was expected that the anatomical characters which usually occur in plants living in insolated areas would be more strongly marked in the members of the dry flora than in the plants of the wet patanas, but it was found that the latter exhibited highly developed xerophilous features. The dwarfing effect of the wind was recognised as an important influence and emphasis was laid on the lowering of the functional activity of the roots by the humic acids in the soil.

Pearson's early training was exceptionally good as a preparation for his later activities at the Cape. Familiar with modern laboratory methods, he was also a capable field botanist, and at Kew he learnt the business of a systematist. His broad outlook, his wide knowledge of botanical literature,

and his enthusiasm for research enabled him to compress into a short scientific career more useful work than most botanists would have been able to accomplish.

Most of the papers published during his official connection with the Royal Gardens deal with systematic botany; he contributed accounts of several new species to the 'Icones Plantarum,' and in collaboration with Mr. Botting Hemsley, described collections of plants made by Dr. Sven Hedin and Sir Martin Conway from Central Asia and the Bolivian Andes respectively. He assisted Mr. Hemsley in his comprehensive account of the flora of Tibet. He wrote an article on the flora of Palestine for the 'Encyclopædia Biblica,' contributed a section on the Verbenaceæ to the 'Flora Capensis,' and one on the Thymelæaceæ to the 'Flora of Tropical Africa.' At the suggestion of Sir William Thiselton-Dyer, he investigated the morphology and functions of the double pitchers of four species of Dischidia. He showed that the outer pitcher is part of a modified leaf and corresponds morphologically to the single pitcher of *Dischidia Rafflesiana*, the inner pitcher being formed from the inflexed apical lobe of the leaf. Good evidence is brought forward in favour of the view that the soil in the pitchers is carried there by ants, and that they use the inner pitchers as a refuge during temporary floodings of the larger outer pitchers. This piece of work is surprisingly good considering that the material examined consisted of herbarium specimens.

The most important contributions made by Pearson to Morphological Botany are those on the Gnetales. His first visit to the Welwitschia country was in January, 1904, but the Herero rebellion seriously interfered with his field observations. In a paper published in 1906 in the 'Philosophical Transactions,' he describes the climatic and physical conditions of the German territory, where he received invaluable help from the Imperial Consul-General, and gives an interesting account of his observations on the habit and habitat of Welwitschia. He expresses the opinion that the period, 70–100 years, usually assigned as the duration of life, is much too short, and confirms Marloth's statement that Welwitschia is diœcious; he considers that the cones are probably pollinated by insects, and records the frequent occurrence of the beetle, *Odontopus sexpunctulatus*, though it was not until a subsequent visit that he obtained evidence convincing him that *Odontopus* is the pollinating agent. Much new information is given as to the development of the male and female flowers. Hooker's account of the ovule is generally confirmed and considerably extended. In the earlier stages of development the embryo-sac contains numerous free nuclei; this condition is followed by partial septation, which produces a tissue of multinucleate compartments. He found that, in the upper part of the embryo-sac, each "cell" has 1–2 nuclei, and later as many as 5, while in the lower part of the sac each "cell" has 2–12 nuclei. The "cells" with 2–5 nuclei produce prothallus-tubes which, he shows, contrary to Strasburger's view, cannot be regarded as equivalent to archegonia. He

concludes that the fertile end of the prothallus is more specialised than the corresponding region in Gnetum and that Welwitschia can no longer be placed in the "enormous gap" which separates *Gnetum gnemon* from Ephedra.

A second expedition to Damaraland in 1906–7 enabled Pearson to fill up gaps in the first account, and to confirm or correct his earlier impressions. In the "Further Observations" he discusses the morphology of the inflorescence and flower, particularly with reference to possible affinities to the Bennettitales and the flowering plants. Additional facts are given about pollination. The most interesting questions discussed concern the nature of the tissue in the embryo-sac. The tubes which grow upwards from certain cells in the embryo-sac to meet the descending pollen-tubes are now termed embryo-sac-tubes in preference to prothallus-tubes, because the examination of a more complete series of developmental stages led him to regard the tissue in the macrospore as neither part of the gametophyte nor of the sporophyte, but a distinct organism, which he names the trophophyte. All the free nuclei in the embryo-sac, when septation begins, are believed to be potential gametes. The sterility of the tissue in the lower part of the sac is attributed to the fusion of the nuclei in the multinucleate compartments; this tissue, with nuclei possessing more than the premeiotic number of chromosomes, is a by-product, resulting from the fusion of potentially sexual nuclei, and it agrees with the prothallus of the lower seed plants in providing for the nutrition of the embryo. In Ephedra the endosperm is a true prothallus as in Conifers, but the recognition of the true nature of the Welwitschia prothallus raised the question of the morphology of the tissue in the embryo-sac of Gnetum and the Angiosperms. It was primarily for the purpose of collecting material of *Gnetum africanum* that Pearson organised and, with the assistance of the Percy Sladen Trustees, successfully carried out his important expedition to Angola and other regions in 1908–9. He found in the embryo-sac of Gnetum a differentiation similar to that in Welwitschia; after septation, the compartments in the lower part of the sac contain five or more nuclei, but, in the micropylar portion of the embryo-sac, septation does not occur. Pearson also describes the germination of the microspore of Welwitschia and the development and structure of the pro-embryo. His investigations clearly demonstrate that Welwitschia and Gnetum are related to one another more closely than either is related to Ephedra, a result which "might be expected from the geographical distribution of the three genera."

By his own researches, Pearson not only very considerably extended our knowledge of Welwitschia and Gnetum, but his thorough and critical investigations have thrown much new light on questions of general interest, the nature of the tissue in the embryo-sacs of Gnetum and Welwitschia and of the Angiosperms, the morphology of the flowers and inflorescences, the embryogeny, and other subjects of morphological and biological interest. Pearson's generous distribution of material to other botanists enabled them

to benefit by his expeditions, and led to the publication of important results obtained from research along other lines of enquiry than those which appealed more particularly to himself. The rapidity with which he dealt with the material collected on several expeditions and the thoroughness of the work compel admiration, especially when one remembers the small amount of leisure at his disposal and the ever-widening range of his activities. Pearson handled the difficult problems suggested in the course of his researches with remarkable skill and, in dealing with the criticisms of other botanists, he always preserved a true sense of proportion and consistently adopted a fair-minded and scientific attitude.

In reviewing Pearson's scientific life there is a danger of underrating the general botanical value of his exploration work in Damaraland, Namaqualand, Angola, Bushmanland, and other regions. His researches into the morphology and phylogeny of Welwitschia and Gnetum naturally occupy a prominent place in an account of his achievements, and his determination to obtain material of these plants was a primary motive of some of his expeditions. The Gnetales were not by any means the only objective. He devoted himself with extraordinary energy to the investigation in the widest sense of the vegetation of the countries through which he travelled. As he fully recognised, the excellent results of his botanical expeditions were in part due to the generous contributions by the Trustees of the Percy Sladen Fund, the Government Grant Committee of the Royal Society, and other bodies. He made large collections of herbarium specimens and supplied the Royal Gardens, Kew, the Garden of his old University, and other centres with many living plants. As his own published papers show—as well as those of other botanists entrusted with the description of his material—he discovered many new plants and accumulated a large body of facts bearing on the distribution and ecology of South African vegetation. The dedication of the volume of the 'Botanical Magazine' for 1914 to Pearson is a graceful and well-earned tribute to his success as an explorer.

Pearson visited Damaraland four times; on the first three occasions he was hospitably entertained by the German officials and on the last visit at the beginning of 1916 he was the guest of the Headquarters Staff of the Union Forces. Pearson had a strong desire to take some share in the war and offered his services to the Cape Government; his last expedition was, in some degree, war-work, as General Botha encouraged him to make a survey of the recently acquired territory. In a letter written in April, 1915, he said: "I have felt a little easier in my mind since I volunteered for local defence. I am now enrolled as a mounted infantryman, my official title being Trooper Pearson, which gives me some measure of satisfaction."

In letters during his travels he spoke of the fascination of following the changes in the vegetation in the course of a long journey and from time to time referred to progress made in the solution of many interesting phytogeographical problems. Had he lived a few years longer there is no doubt he would have worked up his material into a connected whole and, knowing how

well qualified he was by training and ability to see things in their true perspective, one is able to realise to some extent how valuable such a digest of his knowledge and mature experience would have been.

During an enforced halt in Namaqualand Pearson made observations on the internal temperature of *Euphorbia virosa* and *Aloe dichotoma.* He found that Euphorbia, with its large-chambered pith, responds more quickly than the Aloe to changes in the external temperature and attains higher maxima. He also investigated the effects of wounding: in Aloe the lowering of the internal temperature is due to evaporation at the surface of water conducted through the water-laden xylem, while in Euphorbia the lowering is due in part to surface evaporation but also to the expansion of gases imprisoned in the pith.

The accounts of travels published in the 'Geographical Journal,' the 'Kew Bulletin,' the 'Gardeners' Chronicle' and elsewhere are admirably written and contain much that is of general interest. Pearson took a prominent part in founding the Botanical Society of South Africa, and in 1915 under his editorship the Cambridge University Press published the first part of the 'Annals of the Bolus Herbarium,' a periodical devoted primarily to work inspired directly or indirectly by Dr. Bolus. Pearson was deeply interested in the economic aspect of his subject and illustrated not only by precept but by example the value of botanical research in relation to agricultural science. With characteristic energy he undertook to attack the "Problem of the Witchweed" with which the Department of Agriculture was faced. The Witchweed—*Striga lutea,* a scrophulariaceous plant—is a root parasite, known by many local names, *e.g.*, the Rooibloem, Isona, Mealie gift, etc., which attacks maize crops and causes considerable damage. It is probably a native of South Africa, and its dispersal has been largely determined by the extension of maize cultivation. Pearson, with the assistance of one of his pupils, studied the details of the haustorial structures and their connection with the host; he also investigated the germination and distribution of the small seeds and made many experimental trials of different methods of dealing with the pest. Previous attempts to germinate the seeds of Striga had been unsuccessful, and Pearson proved that germination occurs only in the presence of the host-plant. His results have not only a scientific value, but they enabled him to draw up a number of practical instructions for the treatment of crops and for the prevention of the disease.

It was for many years Pearson's ambition to bring about the institution of a National Botanic Garden: he felt that the Municipal Garden of Cape Town, first established as a Botanic Garden in 1848, was wholly inadequate and unworthy of a country second to none in the richness of its sub-tropical vegetation. Many botanists, especially visitors from Europe, had expressed disappointment with the existing state of affairs and deplored the absence of a State Garden comparable with those in other parts of the Empire. The task of influencing public opinion, overcoming misunderstandings, and of enlisting the active co-operation of those in authority was one requiring

both tact and patience, qualities with which Pearson was fortunately liberally endowed. Though diffident and retiring, he had the courage of his convictions; he was deservedly popular and thoroughly trusted; his one aim was to benefit the country of his adoption and the science which he loved.

In his presidential address to the Botanical Section of the South African Association for the Advancement of Science he stated the case for the establishment of a National Garden with admirable wisdom and foresight; he dwelt on the neglect of the native plants, and pointed out that more South African species are cultivated in European gardens than at the Cape. He emphasized the economic advantages to be gained from the cultivation of indigenous plants, the discovery of new sources of fibre, rubber, drugs, and other products; he also urged the importance of adding to the Garden a National Herbarium, a Museum of Economic Botany, a Library, and Research laboratories.

The Prime Minister (General Botha) received a deputation in April, 1912, and Sir Lionel Phillips warmly espoused the cause of a National Garden in the House of Assembly on May 6, 1913, when it was unanimously resolved to establish a State Garden at Kirstenbosch. The Kirstenbosch estate, bequeathed to the nation by Cecil Rhodes, comprising nearly 400 acres, was handed over to Trustees and Prof. Pearson was appointed Honorary Director. Work began on August 1, 1913. It is an admirable site, and the undulating ground on the eastern slope of Table Mountain, which ranges from 60 to 1500 feet above sea-level, supports a rich native flora, including a fine forest of silver trees (*Leucadendron argenteum*). Pearson's aim was at last realised and, though he modestly disclaimed more than a comparatively subordinate share in its foundation, he is generally regarded in South Africa as the founder of the garden. It was his tactful persistence and his faculty of communicating his enthusiasm to others that led those in authority to give definite expression to his wishes. During his brief tenure of the Directorship, he gave himself unsparingly to the work of construction, and the impression left on my mind, after a visit of a few hours in July, 1914, was that, under his guidance, the Kirstenbosch Garden would in course of time rival the best gardens in the world.

The author (W. Duncan Baxter) of an In Memoriam article in 'The Cape' for November 10, 1916, writes: "It is Kirstenbosch that will feel his loss most. During the last three years he had devoted himself heart and soul to that great undertaking. He saw the importance and possibilities of the starting of the National Botanic Gardens there, and, under his most skilful and efficient direction, the gardens have progressed in a wonderful way and the foundation of all their future scientific work has been well laid. All his spare time was devoted gratuitously to the work, and his genial enthusiasm infected everybody who came in contact with him. He rests in the spot he loved so well, the Cycad Hill facing the mountain and overlooking the glen. What more suitable spot could have been chosen for the resting place of the founder of Kirstenbosch? . . . The best memorial

that can be raised to him is to see that his work at Kirstenbosch is carried on, and the National Botanic Gardens made what he pictured them in his mind's eye. That is the way to perpetuate his memory, for, as long as Kirstenbosch exists, there will be linked with it the name of its founder—the scholar and gentleman, Harold Pearson."

A. C. S.

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ERRATUM.

Pages 364 and 366—Figs. 1 and 2 are misplaced and should be transposed.

END OF THE EIGHTY-NINTH VOLUME (SERIES B).

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